Federation Proceedings

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NOTES ON THE THIRTY-SIXTH ANNUAL MEETING OF THE FEDERATION

New York City, April 14–18, 1952

THE THIRTY-SIXTH ANNUAL MEETING of the Federation was held in New York City, April 14–18, 1952. The headquarters hotels were the Statler and the New Yorker, and scientific sessions were held in the hotels Statler, New Yorker, McAlpin and Governor Clinton and the Manhattan Center. Dr. D. B. Dill, Past-President of the American Physiological Society, was Chairman of the Executive Committee and presided at the Joint Session of the Federation, which was held in Manhattan Center, Tuesday evening, April 15th.

Scientific sessions of the six constituent Societies began on Tuesday morning, April 15th, and continued through Friday afternoon, April 18th. One hundred and forty-nine sessions were held, at which 1510 papers were presented and 161 were read by title. Three papers were presented at the Joint Session and were recorded for broadcast by the Voice of America. The motion picture session on Monday evening, April 14th, showed seven films. Symposia and panel discussion programs were arranged by the American Physiological Society, the American Society for Pharmacology and Experimental Therapeutics, the American Institute of Nutrition and the American Association of Immunologists. During the course of the Federation meeting three Societies scheduled dinners, and various other groups arranged special functions.

The total attendance at the New York City meeting was 6450. There were seventy-one industrial exhibits shown, and eighteen member and institutional exhibits.

FEDERATION CONSTITUTION

At the 1951 Cleveland meeting of the Executive Committee certain minor changes were recommended in the proposed Constitution. The document, as approved at the January 1952 meeting of the Executive Committee, was referred to the six constituent Societies for action at the New York meeting. It was ratified, as submitted, by all of the Societies and by the Executive Committee, and became effective April 17, 1952.

The Constitution vests the management of the Federation in a Federation Board on which each Society is represented by the President, the Secretary, and the immediate Past-President; the Chairmanship of the Board is to be held in turn by the Past-Presidents of the constituent Societies, in order of seniority in the Federation.

The full text of the Federation Constitution and Bylaws will be printed in the December 1952 issue of *Federation Proceedings*.

EXECUTIVE COMMITTEE ACTIONS

1. Two new Federation committees were created: *1*) An advisory committee to the National Society for Medical Research, composed of six members, one from each of the constituent Societies, Chairmanship of which will be held for a three-year term by Dr. F. S. Robscheit-Robbins; *2*) a committee to work in liaison with the Canadian committee in charge of the XIX International Physiological Congress, composed of seven members (one member from each of the constituent Societies and Dr. E. M. Landis, *Chairman*).

2. Plans were confirmed for the 1953 meeting in Chicago, April 6–10. The American Society of Biological Chemists will be senior Society at this meeting, and Dr. Vincent du Vigneaud, as Past-President of that Society, will serve as Chairman of the Federation Board. Dr. E. H. Stotz will be Chairman of the Secretaries' Committee. Other future meetings have been set for Atlantic City in 1954 and 1956, and Los Angeles in 1955.

3. The Federation assessment for the year 1952–53 was set at $3 per member of each constituent Society.

4. Dr. M. O. Lee was reappointed Federation Secretary.

5. It was agreed that the Federation affiliation with the American Association for the Advancement of Science should in future be through Section N (Medical Sciences) rather than through Section FG (Zoological and Botanical Sciences).

6. The Executive Committee approved continued participation through its representatives in the work of the Science Manpower Commission.

PLACEMENT SERVICE

A total of 332 candidates registered with the Placement Service at the New York City meeting, distributed in the fields of the Societies as follows: Physiology, 105; Biochemistry, 152; Pharmacology, 44; Pathology, 7; Nutrition, 6; Immunology, 18. This was an increase of 123, or 59%, over the registration at the Cleveland meeting. There were 577 interviews scheduled during the meeting.

CORRECTIONS AND ADDITIONS TO MARCH ISSUE

Page 24. CHASE AND BRIGHAM, *next to last line of abstract:* The word "more" should read "less."

Page 25. L. A. COHEN. *Present address of author:* University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Page 69. The following abstract was omitted.

Higher centers of statokinetic regulation (motion picture). W. R. HESS. *Univ. of Zurich, Zurich, Switzerland.*

Statokinetic reflexes emanating from the metencephalon and mesencephalon, as described by MAGNUS AND RADEMAKER, are well known. The film shows reflexes of a higher order originating from the diencephalon. In the non-anesthetized animal, the position of the eyes, head and trunk is at all times under the constant control of a system of highly differentiated mesencephalic and diencephalic reflexes. The reflex system thus provides a dynamic basis for voluntary movement, the governing impulses being supplied mainly by the vestibular apparatus and proprioceptive mechanisms.

Page 72. HOLBURN, CARROLL AND TOCANTINS, *14th line of abstract:* The sentence beginning on this line should read: "The mean anticephalin activity/ml. plasma in 34 normal plasmas was 14.0 U ± 6.5 (S.D.) (range 1.0–27.0). In 14 determinations on 4 hemophilics the mean was 56.0 U ± 14.6 (S.D.) (range 26.85–85.2)."

Page 77. EDMUND JACOBSON, *16th line of abstract:* The term "mv." should read "microvolts."

Page 95. LIM, NOVAK AND WALSH, *18th line of abstract:* The sentence beginning on this line should read, "Methantheline bromide and other cholinolytic agents, applied topically . . ."

Page 95. GILBERT LING, *11th line from end of abstract:* The values given should read: "Rb^+, 2.28 Å; K^+, 3.88 Å; Cs^+, 4.40 Å; Na^+, 5.62 Å; Li^+, 7.18 Å."

Page 105. MELAMPY AND PORTER, *line 8, line 10:* instead of "dry" read "fresh"; *line 14:* the sentence beginning "Fructose was absent . . ." should be deleted.

Page 167. I. H. WAGMAN, *8th line from end of abstract:* Instead of "58 inches," read "68 inches."

Page 304. WAINIO, EICHEL AND COOPERSTEIN, *authors:* add N. H. COY AND Q. OCHS; *laboratory of origin:* add *Squibb Inst. for Med. Research, New Brunswick, N. J.; line 8:* the term "cathode" should be changed to "anode."

Page 350. HELEN TREDWAY GRAHAM *et al.*, *authors:* name of fourth author should read ANSEL R. MARKS; *7th line from end of abstract:* 5000,000 should read 500,000; *end of abstract,* add the line: "Supported in part by USPHS grant H 156."

Page 389. F. W. SCHUELER, *10th line:* Instead of "P.F. = +.089," read "P.F. = +0.89"; *18th line:* instead of "−.068" read "−0.68."

Page 434. The following abstract was omitted.

Corticotropin preparations which, when given intramuscularly, exhibit human activity several times that anticipated from animal bioassay. WILLIAM Q. WOLFSON AND WILLIAM D. ROBINSON (introduced by REUBEN L. KAHN). *Rackham Arthritis Research Unit, Dept. of Internal Medicine, Univ. of Michigan, Ann Arbor.*

Crude corticotropins from various sources have generally shown good parallelism between animal assay and human potency. However, ACTX-corticotropins (Wilson and Armour), chromatographically fractionated high-potency preparations, consistently show human activity far greater than expected from animal bioassay. When given intramuscularly q.6.h. in light gelatin, ACTX-corticotropin showed human metabolic and clinical activity more than 650% of aqueous ACTH-corticotropin (U.S.P. corticotropin) similarly administered. In fairly extensive metabolic and clinical studies, long-acting ACTX-corticotropins have been found 3–4 times as effective as crude corticotropin in similar menstrua. The pattern of metabolic effects elicited by ACTX-corticotropins and crude corticotropin has appeared identical. These findings confirm those of RABEN, ROSENBERG, WESTERMEYER AND ASTWOOD (*J.A.M.A.* 148: 844, 1952) with similar materials. Since increased activity of ACTX-corticotropin more than compensates for losses in its preparation, it will probably become available for general use. This may require replacing the provisional U.S.P. corticotropin assay by one more closely paralleling clinical effectiveness. Certain observations suggest the heightened intramuscular activity of ACTX-corticotropin may result from decreased extravascular inactivation.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Symposium on Fate and Mechanism of Action of the Barbiturates[1]

Chairman: M. H. SEEVERS

THE DISTRIBUTION AND FATE OF DIALKYLBARBITURATES[2]

E. W. MAYNERT

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York City

ALTHOUGH it was originally proposed that this paper should be concerned with the localization and fate of all barbiturates except those containing sulfur, the discussion will be confined to the common dialkyl derivatives of barbituric acid. This arbitrary restriction of the topic seems justified, because most of the newer research on barbiturates has been concerned with the dialkyl derivatives. Furthermore, all the published information on the distribution and fate of barbiturates containing unsaturated side-chains or N-alkyl groups has been previously reviewed (1). Recent work on the metabolic fate of dialkylbarbiturates has been rather extensive, and the present occasion offers a good opportunity to summarize some of the latest findings, to try to fit them in their proper places, and to indicate what remains to be done in this field.

METABOLIC FATE

If one considers the chemical structure of a dialkylbarbiturate, he will see that there are two ways in which the drug could be detoxified. One or both of the alkyl side-chains could be oxidized, or the barbituric acid ring could be hydrolyzed. One scheme of hydrolysis is shown in figure 1. The barbituric acid is converted to a malonuric acid which is subject to the loss of carbon dioxide to yield a dialkylacetyl urea; hydrolysis then proceeds to give a dialkylacetamide. These reactions are known to occur *in vitro* when barbiturates are treated with alkali. In 1933 Shonle and his colleagues (2) suggested that these reactions also occur *in vivo* as the primary steps in the detoxification of pentobarbital and Amytal. Furthermore, they suggested that compounds like the acetamide and the acetyl urea would be completely metabolized to ammonia, carbon dioxide and water. This hypothesis would, of course, explain their failure to detect either the drugs or metabolities of them in the urine.

The availability of isotopic methods made it possible to answer the question of metabolic hydrolysis of the barbituric acid ring. Pentobarbital and Amytal were labeled with N^{15} and administered in anesthetic doses to dogs. Then determination of the amounts of isotope in the urinary ammonia and urea fractions measured the extent to which the nitrogen of the drugs was converted to ammonia in the body. The results (3, 4, 5) were that less than 10 per cent of the nitrogen of either drug is metabolized to ammonia. The next step was the synthesis (6) of the theoretical hydrolytic products of pentobarbital and Amytal—those corresponding to the compounds in figure 1 plus certain others like the malonamides—and employment of the isotope dilution technic to determine whether these compounds are excreted in urine as end-products of the drugs. The findings were that none of these compounds occurs in the urine of dogs after the administration of pentobarbital or Amytal. Thus, hydrolysis was ruled out as the mode of detoxification for these drugs, and it became clear that the oxidation of the alkyl side-chains was involved.

The problem of the elucidation of the chemical structures of the metabolites might have been attacked in a similar manner by synthesis of oxidative derivatives of the drugs for use in the

[1] Fall Meeting, Omaha, Nebraska, October 15–17, 1951.

[2] Experimental work by the author and his colleagues presented here was supported by a research grant from the National Institutes of Health, Public Health Service.

isotope dilution technic. However, this approach was unattractive for several reasons. First, the number of theoretical oxidative products of pentobarbital and Amytal is quite large; this is true even if one chooses to ignore the existence of stereoisomerism. Secondly, the synthesis of most of these compounds would be quite difficult. Finally, no evidence was available to exclude the possibility of partial hydrolysis or conjugation occurring subsequent to oxidation. At this point there was clearly only one way to approach the problem of metabolic fate, and that was to resort to the ordinary methods of chemical isolation using information gained from the isotopic tag as a guide.

If one subjects a sample of urine from dogs after the administration of isotopic pentobarbital to continuous extraction with ether, ethyl acetate or butanol, he will find that about 65 per cent of the isotope can be transferred to the organic solvents. The remainder of the isotope cannot be extracted with any common solvent. This non-extractable fraction will be discussed later. It was on the extractable metabolites that attention was first focused.

From ether extracts of large quantities of urine from dogs after the administration of ordinary, unlabeled pentobarbital it was eventually possible to isolate two pure metabolites of the drug. These substances did not possess any anesthetic or hypnotic activity in mice even after large doses. Both compounds had an elementary composition corresponding to pentobarbital with one additional oxygen atom. Their ultraviolet spectra established the presence of the barbituric acid ring. Infrared specta indicated the presence of a hydroxyl group in each metabolite which was confirmed by the preparation of crystalline acetates (fig. 2). Positive iodoform reactions demonstrated the presence of the CH_3CHOH-group. Finally, treatment of each metabolite with concentrated sulfuric acid cleaved the secondary chain to yield ethyl barbituric acid; this reaction proved conclusively that the CH_3CHOH-group was derived from the 1-methylbutyl group and not from the ethyl group. One of the compounds was strongly dextrorotatory; the other, weakly levorotatory. Hence, the metabolites must be diastereoisomers of ethyl (3-hydroxy-1-methylbutyl)barbituric acid (7, 8). It will be noted that this structure has two asymmetric carbon atoms; thus diastereoisomerism would be predicted.

The quantitative determination of the metabolites in urine was made with the isotope dilution technic. In order to guard against erroneous results due to the coprecipitation of other labeled compounds in the urine, the products from the isotope dilution experiments were analyzed not only as the alcohols but also as their acetyl derivatives. The findings were that dogs excrete 33 to 35 per cent of an anesthetic dose of pentobarbital as the levorotatory alcohol and 15 per cent as the dextrorotatory alcohol. These same alcoholic end-products are also excreted by man after the

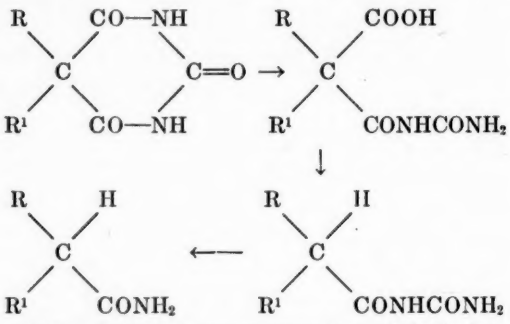

Fig. 1. SHONLE'S HYPOTHESIS for metabolic fate of pentobarbital and Amytal.

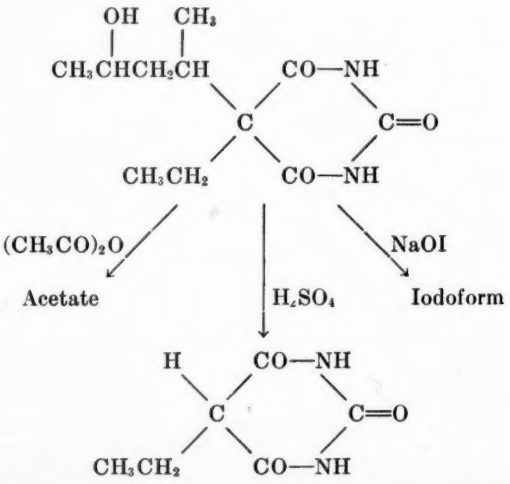

Fig. 2. STRUCTURE PROOF for metabolites of pentobarbital.

administration of therapeutic doses of the drug. However, the two compounds do not occur in the same ratio. Exact figures for the excretion in man are not available at this time. The problem of obtaining such data is complicated by the fact that the metabolites are excreted very slowly by man compared with the dog—several days compared with one day. This, of course, leads to very low concentrations of N^{15} in urine and thereby greatly increases the errors in estimating the excretion of isotope.

The isotope dilution technic is at best a very laborious and time-consuming method. The determination of the alcoholic end-products of pentobarbital presented an extra difficulty. Large amounts of the pure metabolites had to be isolated before the dilution experiments could be started. However, the use of the method was justified here, because it is probably the only method which could be used for the determination of the individual isomers. Although the isomers differ in solubility, their partition coefficients among the common solvents are almost identical. Hence, it would be almost impossible

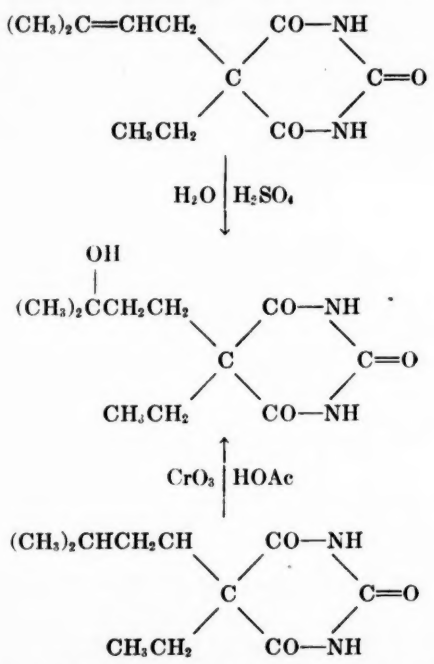

Fig. 3. Two syntheses of metabolite of Amytal.

to devise analytical methods for the individual isomers based on extraction alone.

It is apparent that there is at least one other extractable metabolite of pentobarbital; 65 per cent of the isotope in the urine is extractable and yet only 50 per cent can be accounted for as the diastereoisomeric alcohols. So far, the compound or compounds which comprise this 15 per cent have not been determined. Perhaps there are small amounts of many different compounds.

The labeled metabolites of pentobarbital in urine which resist extraction appear to be glucuronic acid conjugates, at least in the dog (9). The evidence comes from three independent methods. First, in carefully controlled experiments, there is an increase in the excretion of glucuronic acid after the administration of pentobarbital. Secondly, the non-extractable N^{15} in urine can be precipitated with basic lead acetate; this is, of course, an established reagent for the isolation of glucuronic acid conjugates. Finally, the non-extractable isotopic compounds can be converted to extractable compounds by the action of liver glucuronidase. Taken together these observations make a fairly strong argument for the existence of glucuronic acid conjugates as end-products of the metabolism of pentobarbital. *A priori*, it would seem likely that the glucuronic acid is linked to a hydroxyl group in the 1-methylbutyl side-chain of the drug. However, whether the alcohols involved are the metabolites described earlier is still a moot question.

I would like to pass now to a discussion of what has been learned about the metabolic fate of some of the other dialkyl barbiturates. It was possible to isolate only one end-product of Amytal. The compound was again an alcoholic derivative of the drug but was optically inactive. It resembled the end-products of pentobarbital in that the hydroxyl group was located at the γ-carbon atom of the longer chain. The structure was proved by two syntheses (fig. 3). The first involved the addition of water according to Markownikoff's rule to γ,γ-dimethylallyl, ethyl barbituric acid. The second method was based on the greater ease of oxidizability of tertiary carbon atoms; treatment of Amytal with chromic acid at room temperature gave the metabolite in comparatively good yield. The quantitative aspects of the excretion of this compound were investigated with the isotope dilution technic. The findings were that dogs excrete 67 per cent of an anesthetic dose of Amytal in the form of the γ-hydroxy derivative (10). This high yield signifies that the metabolic fate of Amytal is now known more completely than that of any other barbiturate except barbital.

The metabolite of Amytal possesses no asymmetric carbon atoms; hence there is no possibility for optical isomerism. For the metabolites of pentobarbital optical activity was a necessity, because the drug itself has an asymmetric carbon atom. It was of interest to determine whether the metabolic introduction of a hydroxyl group resulting in a molecule with one asymmetric carbon atom yields an optically active or a racemic product. The compound selected for study was Neonal, *n*-butyl, ethylbarbituric acid. Only one

metabolite of this drug could be isolated. The product was an alcoholic derivative, and again the hydroxyl group was proved to be located at the γ-carbon atom (fig. 4). Since the metabolite was optically inactive, its structure was confirmed by synthesis (10).

At this juncture it seems appropriate to comment on the present state of knowledge of the biological oxidation of alkyl groups. The number of compounds containing alkyl groups which have been studied for elucidation of metabolic fate is limited, but almost without exception the findings have conformed with a pattern involving oxidation at the end of the alkyl chains (11, 12). Such oxidation is called ω- or terminal methyl oxidation. Usually the product is a carboxylic acid which may then be subject to the more familiar β-oxidation of fatty acids. Beta-oxidation of a fatty acid, of course, leads to another acid with two fewer carbon atoms, and this process may be repeated several times. The alcoholic metabolites of pentobarbital, Amytal, and Neonal which have been described reflect an unusual pattern of biological oxidation. The only precedent in the literature for this type of oxidation was reported by Fieser (13) in his studies on the 2-alkylnaphthoquinone antimalarials. He described the γ-oxidation of an isoamyl chain like that encountered in Amytal. The new examples plus the one by Fieser should be sufficient to emphasize the necessity for considering patterns of oxidation other than ω- and β-oxidation.

All of the examples of non-terminal oxidation of alkyl groups to alcohols mentioned above involved chains which are four carbon atoms in length, and in every case oxidation occurred at the γ-carbon atom. From these studies it was not clear whether the pattern should be regarded as γ-oxidation (i.e. oxidation at a site three carbon atoms from the ring) or (ω-1)-oxidation (i.e oxidation at a site one carbon atom from the end of the chain) or whether the pattern was relatively nonspecific. In order to answer this question it was necessary to study a dialkyl barbiturate with a longer chain. For this purpose Ortal, ethyl, *n*-hexylbarbituric acid was selected. By counter-current distribution it was possible to demonstrate the presence of four different metabolites of the drug (9) but structures have been assigned to only three of these (see figure 5). Two of the compounds were acids. One of these was the ω-oxidation product of Ortal; the other, the corresponding β-oxidation product. The third metabolite was a methyl ketone, since it gave a positive iodoform test.

Until the structure of the fourth metabolite of Ortal is elucidated, conclusions about the specificity of non-terminal oxidation must be held in abeyance. However, the characterization of the ketonic metabolite does suggest that the (ω-1)- rather than the γ-carbon atom is the important site for oxidation. Naturally, much more work is necessary on this question. It should be pointed out that the detection of a ketonic rather than an alcoholic metabolite was not at all unexpected. It is perhaps more surprising that ketones were not encountered as end-products of pentobarbital and Neonal, inasmuch as secondary alcohols and ketones are often interconvertible *in vivo*.

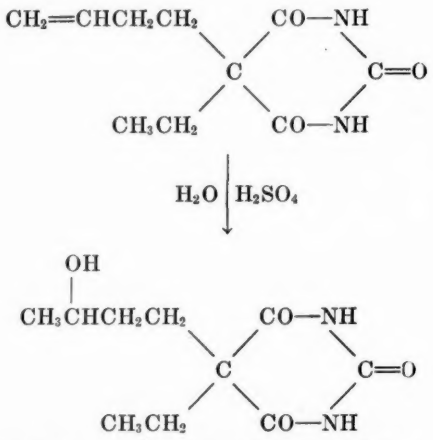

Fig. 4. SYNTHESIS of metabolite of Neonal.

From the data available at present it would appear that non-terminal oxidation is less general than ω-oxidation. As a matter of fact, ω-oxidation is known to occur with the *n*-butyl group when attached to the benzene ring and with the 1-methylbutyl group when attached to the thiobarbituric acid ring. This last observation was made by Brodie *et al.* (14) in a study of the metabolism of thiopental in man. It is rather surprising that the simple replacement of an oxygen in the barbituric acid ring by sulfur leads to a different pattern of biological oxidation. But facts like these emphasize the necessity for caution in reasoning by analogy in problems of metabolic fate.

BIOLOGICAL DISTRIBUTION

Studies on the distribution of barbiturates in the animal body are abundant, but data which

have been obtained by reliable methods are relatively scarce (1). Unfortunately, it has not been recognized by most investigators in this field that barbiturates can be metabolized to compounds which respond in their analytical methods in exactly the same way as the drugs themselves. With methods like the cobalt-color or ultraviolet spectra as they have usually been employed, one cannot distinguish between a solution of a barbiturate and a solution of metabolites of the drug which also contain the barbituric acid ring. Similarly, gross isotopic analysis cannot distinguish between a drug and its metabolites.

Since most of the methods for the determination of barbiturates in tissues and biological fluids have involved extraction of the drugs into an organic solvent, it may be worthwhile to state here a biochemical generality. It has been known for a long time that the metabolites of most drugs differ in their solubility characteristics from the drugs themselves. In almost every case, the metabolites are more soluble in water and less soluble in organic solvents than the original drug. In other words, the metabolites have lower partition coefficients than the drugs. This difference in partition coefficients makes it possible in many cases to select a solvent or a mixture of solvents which, when employed in the proper amount with respect to the biological fluid or tissue, will extract all of the drug but only a negligible amount of its metabolites. This possibility has been particularly appreciated by Brodie and his colleagues who have had gratifying success in the development of specific methods for the determination of numerous drugs. In the course of their work they have developed a very simple technic for gauging the specificity of extractive procedures—the method of comparative partition ratios (15). Counter-current distribution (16) is another method which may be used for examining the specificity of an extraction. Neither of these technics is infallible, but if an extractive procedure satisfies even one of these tests, it is very likely that the method is specific.

This is obviously not the place to review the methods of comparative partition ratios and counter-current distribution. What should be done is to emphasize that at the present time each barbiturate must be considered as a different compound as far as extractive procedures are concerned. This means that specificity must be proved for every barbiturate. Although it is theoretically possible that there is some solvent or solvent mixture which will extract every barbiturate but none of their metabolites, no such solvent has yet been discovered.

One example should suffice to illustrate the necessity for specific methods for each barbiturate. Most investigators have employed chloroform or ether to extract barbiturates from urine or tissues. However, if one extracts a sample of urine (pH 6) collected after the administration of Ortal with an equal volume of chloroform, in addition to the trace of unchanged Ortal present he will extract 60 to 65 per cent of the ketonic metabolite and about 10 per cent of the other extractable metabolites of the drug in the urine. If he extracts another sample of the same urine with an equal volume of ether, he will extract more than 90 per cent of the ketonic metabolite and perhaps 40 to 50 per cent of the other extractable metabolites. From these data one must conclude that chloroform and ether are useless for the determination of Ortal in urine.

Similarly, the possibility of the presence of metabolites must be considered in attempting the determination of barbiturates in tissues, and

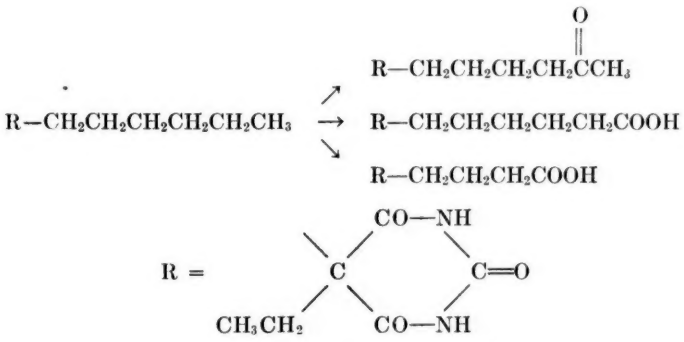

Fig. 5. METABOLITES of Ortal.

the specificity of methods must be established. In the analysis of tissues for drugs there are two factors which probably operate to make the requirements of analytical methods less stringent than if the procedures were to be used for urine. First, most studies of biological distribution are made early after the administration of drug. Naturally, a short time interval between administration and sampling helps to prevent the accumulation of metabolites. Secondly, the end-products of the barbiturates are more polar than the drugs and hence are probably poorly reabsorbed in the renal tubules after filtration (17). This leads to a rate of excretion for the metabolites which is rapid compared with that of the drugs. Thus, in many cases there is probably not much accumulation of metabolites in the body. However, these are theoretical considerations; they do not annul the necessity for experimental proof of the specificity of analytical methods.

The biological distribution of a drug is determined by two factors. One of these is the capacity of the drug to permeate the capillary and cellular membranes. The other factor can be rather loosely referred to as binding and includes protein binding, localization in fat and perhaps binding to other cellular elements such as nucleic acids. Numerous analyses of most organs of the body suggest that barbiturates enter all cells. However, the drugs vary in the rates at which they are transferred from the blood to cells. The most noteworthy illustration is due to Butler (18), who showed that the long-recognized delay in anesthesia after the intravenous administration of barbital was caused by a slow accumulation of drug in the brain.

Most of the available data on the binding of barbiturates to protein were obtained by Goldbaum and Smith (19) who used human and bovine serum albumin. The binding of barbital is very small—5 per cent or less (17). An increase in the length of alkyl side-chains leads to increased binding; pentobarbital is bound to the extent of about 50 per cent. According to Goldbaum and Smith (20) the binding to tissue proteins in rabbits as determined by ultrafiltration follows the same order: barbital being bound the least, phenobarbital being bound to a somewhat greater extent, and pentobarbital and seconal, the most. As far as differences in tissues are concerned, liver and kidney bind more drug than the other tissues.

The question of the concentration of barbiturates in body fat has been approached in a direct way only by Brodie and coworkers. Particular interest in this matter derives from their demonstration that localization of thiopental in depot fat appears to be concerned with the ultra-short action of thiopental (14). They have reported (21) that pentobarbital is not appreciably concentrated in body fat. From this observation it may be presumed that the other dialkylbarbiturates also lack an affinity for depot fat.

In connection with the distribution of barbiturates in the body the most intriguing question has been whether the drugs are concentrated in particular regions of the central nervous system. For a number of years there was a controversy (22–25) as to whether barbiturates are specifically localized in the diencephalon, where centers regulating the degree of sleepiness or wakefulness are thought to reside. However, until recently only inadequate analytical methods had been applied to the problem. The most accurate data presently available were obtained by the use of barbital labeled with N^{15} (table 1). In this study (26) the employment of gross isotopic methods was legitimate, because barbital is completely stable *in vivo*, at least during the first day after its administration. The data show quite clearly that barbital is not localized in the diencephalon or in any of the other parts of the brain which were examined. Obviously, the information which can be gained from an experiment of this kind is limited. The possible existence of very dissimilar concentrations of drug at tiny centers within the gross anatomical divisions of the brain cannot be removed from consideration. However, there is no necessity for assuming a concentration gradient; the pharmacological effects of the barbiturates can be explained just as easily on the basis of a special sensitivity of certain nerve cells.

TABLE 1. CONCENTRATION OF LABELED BARBITAL IN DIVISIONS OF BRAIN[1]

DOSE	CEREBRAL CORTEX	DIENCEPHALON	MEDULLA AND PONS	CEREBELLUM	SPINAL CORD
mgm/kg.					
25	33	35	49	37	
25	24	27	32	26	
125	150	145	130	140	120
250	290	260	280	280	270

[1] Micrograms/gm. tissue.

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PHYSIOLOGICAL DISPOSITION AND CHEMICAL FATE OF THIOBARBITURATES IN THE BODY

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THE DURATION of effect of a volatile anesthetic is determined largely by its rate of excretion across the respiratory epithelium. On the other hand, the duration of the effect of a non-volatile anesthetic such as a barbiturate has been thought to be determined largely by its rate of metabolic transformation. The duration of anesthesia with the barbiturates that were initially available was too long to be acceptable in general anesthesia. Thiopental was introduced as an ultra-short-acting drug; it produces anesthesia of only short duration following the administration of small total doses. However, a patient maintained under prolonged anesthesia by successive injections of thiopental involving a large total dose has a continuing and prolonged narcosis subsequent to the termination of drug dosage. Under such a circumstance, it no longer behaves as an ultra-short-acting drug. This characteristic of thiopental, which has not been understood, is a major limitation on its general utility; it has restricted its unsupplemented use, as a rule, to surgical and obstetrical procedures of relatively short duration. The development of a method[1] for the estimation of thiopental has permitted the systematic study of this rather unusual characteristic of the drug's action.

Figure 1 illustrates a typical example of a plasma concentration-time curve in a man receiving a small single intravenous injection. Plasma concentrations declined rapidly for the first 30 minutes; correspondingly the subject recovered rapidly, awakening in about 15 minutes. The early sharp decline of plasma drug concentration could be interpreted as signifying rapid destruction of the drug, thus explaining the quick recovery from anesthesia. The more gradual decline of the second phase of the plasma concentration curve suggests that the rate of metabolic transformation of the drug is actually slow and that the early sharp decline might reflect rapid initial localization of the drug in tissues. As equilibration between plasma and tissues is established, further decline in plasma levels is then dependent only on rate of metabolism of the drug, which is slow.

More precise information on the rate of transformation of thiopental in man was obtained in studies wherein larger doses were administered. One to four gm.[2] of drug were administered and ample time was allowed for reasonably complete distribution of the drug in the body. Two hours after the administration, thiopental was disappearing from plasma at a uniform rate, indicating that a rough diffusion equilibrium between plasma and tissues had been established; plasma concentration in these circumstances declined only 10 to 15 per cent per hour (fig. 2—typical example) and negligible amounts were excreted in the urine. The decline, then, would appear to reflect the true rate of metabolic transformation of the drug. Clinically, the effects of the drug were in agreement with these analytical findings. Subjects who received the 4 gm. dose slept 4 to 6 hours and on return to consciousness experienced a period of post-anesthetic somnolence lasting 10 hours or longer. The slow decline in the plasma concentrations of thiopental is contrary to the generally accepted belief that thiopental is rapidly transformed in the body. The long-lasting effects after successive doses have been administered to prolong surgical anesthesia are readily explained by the relative stability of thiopental *in vivo*.

The distribution of thiopental in the tissues of dogs given the drug intravenously was then studied to find the reason for the early sharp

[1] The specificity of the method used in these studies was appraised by the technique of comparative distribution ratios; this showed the method to be specific in that the measurement included no metabolic products of the drug (1, 2).

[2] Large doses of thiopental, 3–4 gm., were given by intermittent intravenous injection over a period of about 50 minutes; with these doses efficient artificial respiration with oxygen was maintained by the closed carbon dioxide absorption technique until spontaneous ventilation was thought adequate.

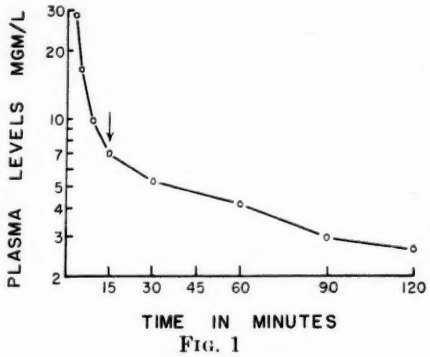


FIG. 1

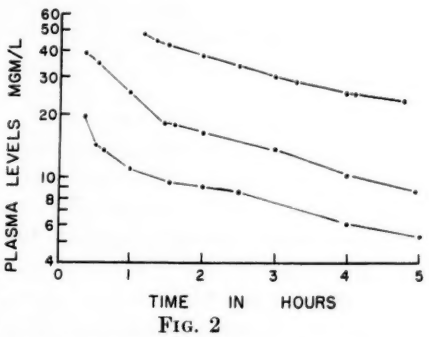


FIG. 2

Fig. 1. PLASMA LEVELS of thiopental after intravenous administration of 0.4 gm. in 2 minutes. Arrow indicates point at which subject awakened.

Fig. 2. PLASMA LEVELS of thiopental after intravenous administration of 1, 2, and 3.8 gm. (lower, middle, and upper curves, respectively). The 2 smaller doses were given in 5 minutes and the larger one over a 50-minute period.

TABLE 1. DISTRIBUTION OF THIOPENTAL IN DOG

TISSUE	CONCENTRATION THIOPENTAL	
	Dog A	*Dog B*
	mg/kg.	*mg/kg.*
Plasma	14.7	19.0
Liver	27.8	
Brain	23.9	
Muscle	22.1	
Heart	18.4	
Kidney	17.6	
Lung	13.6	
Spleen	13.0	
Perirenal fat		222.
Omental fat		192.
Lumbodorsal fat		119.

Tissues were examined 2½ hours after i. v. administration of 65 mg/kg. of drug to *dog A* and 3½ hours after 50 mg/kg. to *dog B*. Thiopental concentrations in fatty tissues are expressed in terms of total lipid content of tissues.

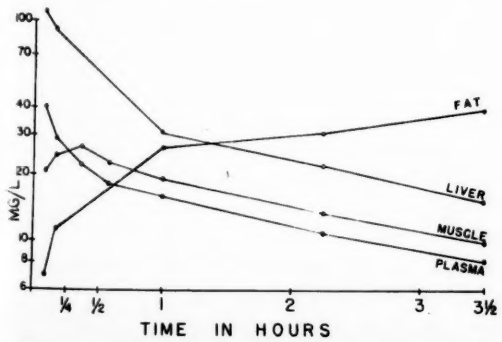


Fig. 3. CONCENTRATIONS of thiopental in various tissues of a dog after intravenous administration of 25 mg/kg.

decline in plasma concentrations and the consequent ultra-short-action of a small dose of thiopental. It is seen (table 1, *Dog A*) that the concentration of anesthetic in most tissues is about the same or somewhat higher than in plasma. The total drug in each tissue was calculated on the basis of published figures for average organ weights in the dog (3). The values account for only about 20 per cent of the thiopental administered. If it were not known from plasma-time concentration curves that thiopental is transformed slowly *in vivo*, these results would seem to indicate that thiopental disappears rapidly from the body. The apparent paradox was resolved when fat was analyzed and found to contain thiopental in concentrations 6 to 12 times greater than those of plasma (table 1, *Dog B*). Analysis of fat biopsies taken from a patient during surgery indicated that this situation also obtains in human beings.

Information was then obtained concerning the rate at which thiopental was distributed between plasma and organ tissues and between these tissues and fat. Thiopental was given intravenously to dogs and samples of plasma, liver, and muscle were taken for analysis at intervals thereafter (fig. 3). The concentrations of thiopental in liver and plasma were found to be maximal immediately following its administration, whereas those in muscle continued to rise for a short time. Liver, muscle, and plasma concentrations then declined rapidly, and in a parallel manner, for about one hour, and then diminished progressively more slowly for the next 2½ to 3 hours. Concentrations in fat, which were negligible at first, rose rapidly for the first

hour, then more gradually and finally reached a maximum in about 3½ hours; following this, the concentrations of the drug in fat diminished gradually.

A comparison of thiopental concentrations in plasma and brain would have been particularly pertinent since the important anesthetic effects of thiopental are exerted on the central nervous system. Technical difficulties were experienced in obtaining repeated biopsies of brain, so comparisons were made instead of drug concentrations in cerebrospinal fluid and plasma. Figure 4 shows drug concentrations in these fluids following thiopental administration to a dog. Plasma concentration levels are expressed in terms of plasma water by correcting for the fraction of drug which is bound to plasma proteins, about 75 per cent. The cerebrospinal fluid concentrations were at first low but rapidly rose almost as high as those of plasma water; they then declined rapidly as the anesthetic left the central nervous system and accumulated in fat. Another pertinent observation is that there is little hindrance to the passage of thiopental across the blood-brain barrier. It seems probable that the initial lag in the cerebrospinal fluid level is a reflection of the time required for the drug to reach the cisterna magna after crossing the blood-brain barrier.

From the above experimental findings thiopental may be regarded as being distributed *in vivo* between two immiscible phases: one, mainly a water phase, consists of blood and organ tissues; the other, fat. The distribution of a weak organic acid like thiopental between an aqueous phase and an immiscible organic phase is largely determined by the relative concentration of undissociated acid. Changes in the pH of the aqueous phase will alter the concentration of undissociated acid and thus alter the partition of the drug between the two phases. It seems probable that the relative concentration of undissociated acid might also determine the distribution of thiopental between the water phase of the body and fat, and that changes in blood pH might alter this distribution.

Dogs which had received thiopental were made acidotic by carbon dioxide inhalation from a Douglas bag containing 10 per cent carbon dioxide and 90 per cent oxygen. Rebreathing of carbon dioxide was continued until the pH of the blood had been suitably altered; the Douglas bag was disconnected and the animal allowed to recover on room air. A decrease of the blood pH to 6.8 was accompanied by a drop in thiopental concentration of about 40 per cent. Recovery was prompt; within 3 minutes after stopping the inhalation of carbon dioxide, the blood pH approximated that of the control period and simultaneously the plasma level of the drug rose sharply. The renal excretion of thiopental was negligible during these experiments; therefore, the fall in plasma concentration during acidosis must represent a transfer of drug from plasma to tissues, presumably fat.

The total amount of thiopental in fat may be calculated by multiplying the total fat by the average concentration of drug in fat. The thiopental in the rest of the body may be approximated by multiplying the fat-free phase (lean body mass) by the average concentration of drug in tissues (taken as 1.5 times the plasma concentration). The lean body mass was calculated from total body water as measured by antipyrine (4); the fat phase is the total body weight minus the lean body mass. On applying these calculations to 2 dogs, about 75 per cent of the anesthetic in the body was shown to be localized in fat after a period of 3 hours.

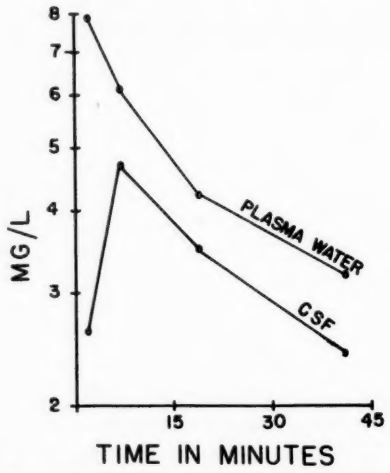


Fig. 4. Concentrations of thiopental in plasma water and cerebrospinal fluid of a dog after intravenous administration of 25 mg/kg.

Certain conclusions can now be drawn concerning the localization of thiopental in fat and its ultra-short-acting characteristics. The drug is ultra-short-acting after a small dose not because it is rapidly metabolized in the body but because it is rapidly localized in fat. The plasma level of anesthetic, after diffusion equilibration between tissues and fat is established, is

below that necessary for anesthesia and the subject awakes soon after the injection. However, after a large dose or after repeated small doses, the resulting equilibrium plasma level is above the anesthetic level and, in fact, is maintained there by the reservoir of drug in the fat. Because of the slow metabolism of thiopental, anesthesia then continues for a considerable period of time.

In the course of these studies, other interesting facts were uncovered which are pertinent to a general study of thiopental and perhaps to other aspects of the general problem of anesthesia and analgesia. These facts bear on the problem of the acute acquisition of drug tolerance.

TABLE 2. PLASMA LEVELS AND CONCURRENT SIGNS OF ANESTHESIA

SUBJECT	DOSE THIOPENTAL	CONJUNCTIVAL REFLEX	EYEBALL MOTION	ORIENTATION[1] (AWAKENING)
	mg/kg.	*mg/l.*	*mg/l.*	*mg/l.*
V	59		32.6	32.6
	36	24.3	22.6	22.6
M	67	27.8	27.8	27.8
	40	19.3	19.3	19.3
G	43	22.8		19.8
	22	14.6		13.2
N	50	27	26.7	26.0
	54	25.8	24.9	24.9
	65	31.6		27.0
	33	16.8	16.8	16.8

Plasma concentrations at time of orientation following the small doses are lower than corresponding levels following the larger doses by an average of 8.6 mg/l. Despite the small number of observations, this difference is statistically highly significant ($P < 0.01$).

[1] Orientation was arbitrarily defined as the ability of the subject to protrude the tongue on spoken demand.

A number of individuals received thiopental in large doses, 43 to 67 mg/kg.; the resulting anesthesia lasted for periods varying from 4 to 5½ hours. The plasma concentrations concurrent with the various signs of diminishing anesthesia are recorded (table 2). In one subject (*N*), thiopental was administered 3 times at weekly intervals in approximately equal doses. It is evident that signs of diminishing anesthesia occurred at approximately the same plasma concentration in each experiment. At least one week following the larger dose of thiopental, the same 4 subjects received smaller doses of the drug, 33 to 40 mg/kg., and slept 1½ to 2½ hours. In each subject, the plasma concentration of the drug declined at about the same rate as in the case of the larger doses. However, the plasma concentrations at which the subjects awoke were considerably lower (table 2). For example, *subject N*, after a dose of 33 mg/kg. of anesthetic, awakened at a plasma concentration of 16.8 mg/l. compared with a waking concentration of 26.0 mg/l. following a dose of 50 mg/kg. The conjunctival reflex and eyeball motions also reappeared at considerably lower plasma levels with the smaller doses of drug.

A phenomenon of acute tolerance is thus evident for barbiturates; after receiving a large dose of thiopental, subjects awake at a plasma level considerably in excess of that which adequately maintains a deep state of anesthesia after a smaller dose. The response of the central nervous system to thiopental appears to depend then on either the initial peak concentration or on the time of exposure of the tissues to the drug. The latter would appear more likely. The time involved in this adaptation is remarkably short—a few hours at most. On the other hand, the tolerance is not persistent; it is no longer than a week and may be of considerably shorter duration. It is probable that a true tissue adaptation is involved; in a manner as yet undetermined the central nervous system develops resistance to the depressant effects of thiopental. The adaptation of the central nervous system to thiopental seems fortunate in view of the stability of the drug in the body. The development of acute tolerance to the drug undoubtedly minimizes the duration of the post-operative narcosis and somnolence.

Pentobarbital, the oxygen analogue of thiopental, is seldom used in man as an anesthetic because of its long duration of action. Studies were made to determine why the simple replacement of sulfur by oxygen causes such a marked change in the duration of its action.

Plasma concentrations of the 2 anesthetics were compared following their intravenous administration to dogs. Before blood samples were taken for analysis sufficient time was allowed for the dog to reach diffusion equilibrium between fat and tissues. It is surprising that pentobarbital metabolizes in dogs more rapidly than thiopental (fig. 5). In man, however, the picture is reversed; thiopental metabolizes more rapidly then pentobarbital (fig. 6). However, the difference in the rate of metabolism of the 2 drugs in

man is not sufficient to explain the ultra-short-action of thiopental relative to pentobarbital.

The distribution of pentobarbital and thiopental was compared in the tissues of dogs that had been given the drugs intravenously. As shown in table 3, the distribution of the 2 drugs is roughly the same in all tissues except fat; some pentobarbital goes into fat, but there is no extensive localization as with thiopental. Fat localization seems to explain the difference in the duration of action of the two anesthetics.

Ultra-short-acting barbiturates besides thiopental, including Surital, Kemithal, and Evipal, are used in anesthesia[3]; of these the first two are thiobarbiturates and the last is an N-methyl oxybarbiturate. These anesthetics, like thiopental, are classified as ultra-short-acting because subjects rapidly recover from the effects of a small dose; however, like thiopental, they are usually used in surgical procedures of relatively short duration since after successive injections of the drugs the resulting narcosis is prolonged.

Studies were made to ascertain whether these anesthetics also undergo slow metabolic transformation in the body and owe their ultra-short-action to rapid localization in fat. Large doses of thiopental, Surital, Kemithal, and Evipal were administered intravenously at weekly intervals to the same person. The plasma concentration-time curve for each drug exhibited 2 distinct phases; an early phase lasting 2 to 3 hours which exhibited a steep slope, and a later phase with a more gradual slope (fig. 7). This suggests that for all 4 drugs the early rapid drop in plasma concentrations is dictated by rapid tissue localization, whereas the subsequent more gradual slope is a reflection of the slow rate of metabolic transformation of the drug. In this particular individual the rate of metabolic transformation of the four drugs was roughly the same, about 10 per cent per hour.

The physiological disposition of Surital, Kemithal, and Evipal was studied to see whether these anesthetics, as with thiopental, are localized in fat. The anesthetics were administered intravenously to dogs, and about 3 hours later the animals were sacrificed and tissues taken for barbiturate analysis. As seen in table 4, fat localization for each of these barbiturates is apparent. It may be concluded from these results

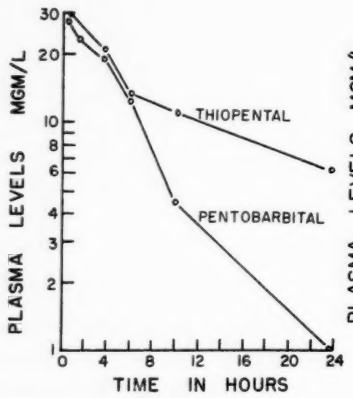


FIG. 5

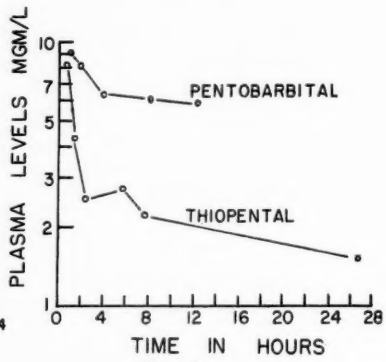


FIG. 6

Fig. 5. COMPARISON of plasma levels of thiopental and pentobarbital in the same dog. Dose, 40 mg/kg.
Fig. 6. COMPARISON of plasma levels of thiopental and pentobarbital in the same man. Dose, 12 mg/kg.

TABLE 3. DISTRIBUTION OF PENTOBARBITAL AND THIOPENTAL IN DOG[1]

TISSUE	RATIO, TISSUE CONC.: PLASMA CONC.	
	Pentobarbital	Thiopental
Liver	1.9	1.9
Brain	1.2	1.6
Muscle	0.8	1.5
Kidney	1.4	1.2
Lung	0.6	0.9
Spleen	1.2	0.9
Fat	1.1	6.2

[1] 3 hours after 40 mg/kg.

[3] Surital is 5-allyl-5(1-methylbutyl)-2-thiobarbituric acid. Kemithal is 5-(2,3-cyclohexenyl)-5-allyl-2-thiobarbituric acid. Evipal is 5-methyl-5-(-1,2-cyclohexenyl)-N-methylbarbituric acid.

that these ultra-short-acting barbiturates are like thiopental in that they are metabolized slowly and are limited in their duration of

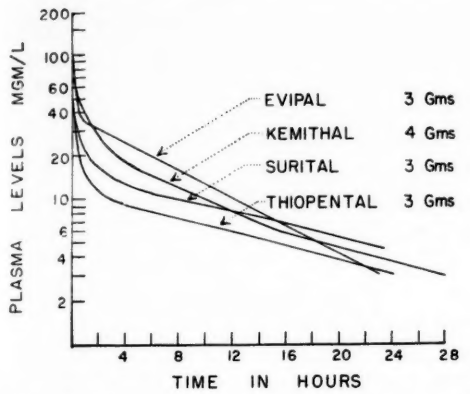


Fig. 7. Comparison of plasma levels of thiopental, Evipal, Kemithal, and Surital in the same man.

Table 4. Distribution of various short-acting barbiturates in dog[1]

Barbiturate	Ratio, tissue conc.: plasma conc.						
	Fat	Liver	Kidney	Lung	Spleen	Brain	Muscle
Thiopental	6.0	1.6	1.2	0.8	0.7	1.2	1.3
Kemithal	8.0	1.3	0.8	0.5			0.8
Surital	8.0	1.6	0.9	0.9	0.7	0.7	1.3
Evipal	3.2	1.3	1.1				0.9

[1] 3 hours after 40 mg/kg.

Table 5. Properties of thiopental metabolite

Ultraviolet Absorption Spectrum

Identical with that of thiopental.

Calculation of molecular wt. from optical density is 273 (Thiopental = 243)

Electrometric Titration

Shows two acid groups: pKa 5.2; pKa 8.2.

Calculation of molecular wt. = 270

Infrared Spectrum

Suggests a carboxyl or an ester group.

Elemental analysis

	Theoretical for a carboxylic acid	Found
Carbon	48.53	48.30
Hydrogen	5.86	6.09
Nitrogen	10.29	10.05
Sulfur	11.77	11.83

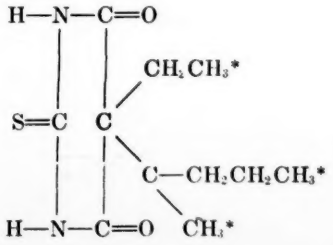
action after a small dose by rapid localization in fat.

The slow rate of metabolism of anesthetic barbiturates makes it clear why the safest use of these drugs should be in the form of intravenous supplementation for other agents. Anesthesia involving the combination of thiopental with nitrous oxide and oxygen should properly and rationally be considered as anesthesia with nitrous oxide and oxygen reinforced with thiopental.

There remains a need for a potent anesthetic which may be used in surgical procedures of long duration without producing prolonged postoperative depression. The behavior of the so-called ultra-short-acting barbiturates suggests that such a drug should be rapidly destroyed in the body rather than accumulated in fat. Studies of the chemical changes that inactivate thiopental were undertaken, therefore, to obtain clues as to in what direction the synthesis of new compounds should proceed.

Thiopental is almost completely metabolized in man; only about 0.3 per cent of an administered dose is excreted unchanged in the urine. Extraction of the urine with ethylene dichloride disclosed the presence of considerable material which, like thiopental, absorbs ultraviolet light at 305 mμ. This material was isolated in pure form by countercurrent distribution. It was found to have no anesthetic activity in mouse or dog. The physical properties of the compound are described in table 5. The ultraviolet absorption curve is identical with that of thiopental, suggesting that the side chain, but not the ring, of thiopental has been altered. Electrometric titration discloses 2 acidic groups, one weak as in thiopental and the other corresponding in strength to a carboxyl group. The molecular weight was calculated both from the optical density and the electrometric titration and was found to be about 271. These results, together with the elemental analysis, indicate that the metabolite is derived from thiopental by oxidation of one of the methyl groups in the alkyl side chains to a carboxyl group; such a derivative would have a molecular weight of 272.

The position of the carboxyl group has not yet been ascertained but could be any one of the 3 positions of thiopental indicated below by an asterisk.

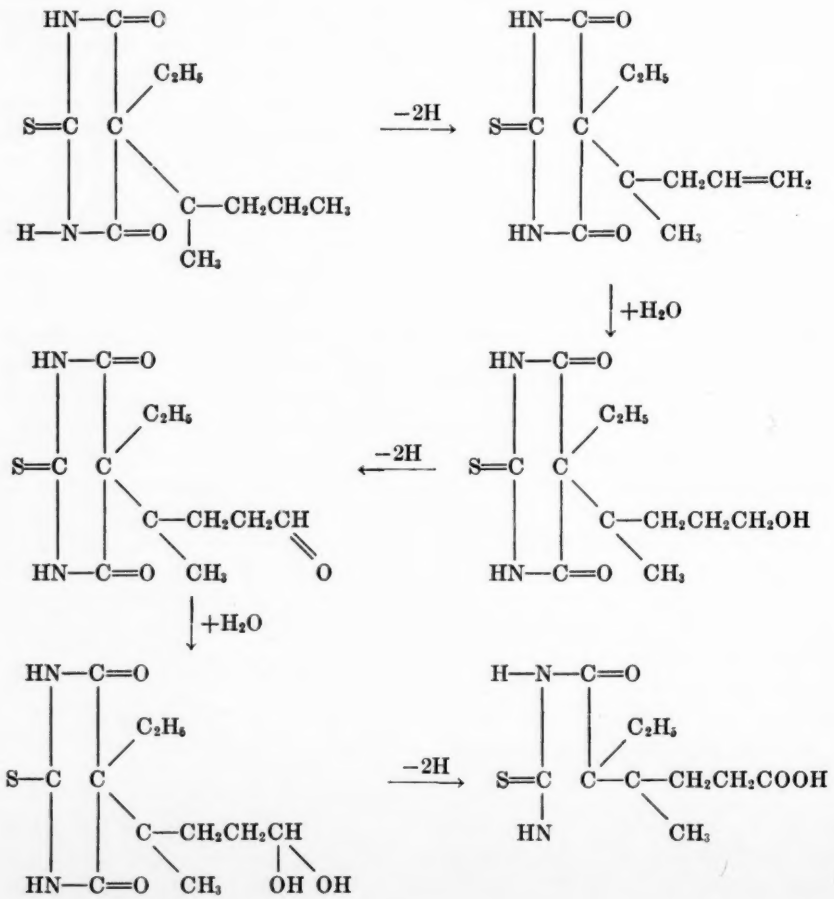


Synthesis of the 3 possible carboxylic acids has been undertaken[4], and comparison of these with the metabolite should permit the definite characization of the metabolite.

The amount of thiopental carboxylic acid excreted in urine was measured after the administration of thiopental. Each of 2 subjects was given 2 gm. of the drug intravenously; the urinary excretion of the acid accounted for about 10 per cent of the administered thiopental. After a 4-gm. dose the urinary excretion of the acid accounted for about 25 per cent. Whether the increased urinary excretion of thiopental carboxylic acid with increase in the amount of thiopental administered reflects a saturation of a system which further metabolizes the acid derivative of thiopental, or saturation of a reabsorption mechanism in the kidney, has not been ascertained.

An interesting example of species difference relative to metabolic transformation of thiopental was found. Thiopental is also most completely transformed in the dog, but only traces of thiopental carboxylic acid are found in the urine. This does not signify that the metabolic route of thiopental differs in dog and man. It is more likely that since the dog transforms the drug so slowly (about 50 per cent in 24 hours) the slow rate of formation of the initial metabolite permits further complete metabolic transformation. Preliminary studies in the dog concerning the fate of thiopental carboxylic acid administered intravenously support the belief that this is so. If thiopental carboxylic acid similarly is unstable in man, it is possible that the transformation of thiopental to this acid is the major, if not the only route of metabolism of the drug.

It is unlikely that thiopental carboxylic acid is formed from thiopental by a simple one-step reaction; it is presumably the end-product of a series of chemical steps. The following series of reactions may be postulated, assuming that the carboxyl group is on the end of the side chain.



[4] E. Horning and H. Wood, National Heart Institute, Bethesda, Maryland.

It may be possible to work out the discrete steps in the oxidation of thiopental by following the fate of some of the postulated intermediates *in vivo*.

Most of the older conjectures concerning the metabolism of barbiturates contain the thought that inactivation involves splitting of the barbituric acid ring. The results on the chemical fate of thiopental, as well as Maynert's work with the oxy-barbiturates (5), indicate that barbiturates can be inactivated by a metabolic transformation which involves oxidation in the side chain. Efforts are being directed to the synthesis of barbiturates with side chains which are easily oxidized *in vivo* to form inactive derivatives.

The work reported here was carried out in collaboration with Eleanore Bernstein, John Burns, Philip A. Lief, Lester Mark, E. M. Papper, and E. A. Rovenstine.

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IN VITRO METABOLISM OF BARBITURATES

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INFORMATION regarding the metabolism of barbiturates by surviving tissue preparations is meager. That the liver and kidneys are involved in the *in vivo* metabolic transformation and elimination of certain of these compounds is well established (1, 2). That other tissues may be involved in some instances, especially in the case of the thiobarbiturates, has been suggested (3). Evidence for some slight degree of metabolism of thiopental by the heart and lungs has been presented previously in a communication from this laboratory (4). In the heart-lung preparation of the dog, after administration of a single dose of the thiobarbiturate, there occur gradually declining plasma levels of the drug (fig. 1). However, this rate of decline is greater when the kidneys are included in the circulation and greater yet in the heart-lung-liver preparation.

In table 1 are summarized the positive results of the *in vitro* experiments reported in the literature. In all instances the tissues were employed in the form of slices or breie. Delmonico (5) in 1940, reported that minced muscle, kidney, brain and liver of the rabbit were capable of metabolizing pentobarbital, thiopental and amobarbital. The validity of such conclusions is questionable since the tissues were incubated for 24 hours, no precautions being taken to avoid or control bacterial contamination and growth. In the same year Martin, Herrlich and Clark (6), employing a macerated preparation of various rat tissues, stated that liver, skeletal muscle and spleen were capable of inactivating hexobarbital. Kidney, brain and blood showed no such activity. The significance of these results is open to question. Tissue preparations were incubated for 10 to 60 minutes at 38° C. in physiological saline containing anywhere from 3.43 to 5.0 per cent drug. No attempt was made to control PH. The amount of drug remaining after incubation was estimated by centrifuging off the particulate material and assaying the supernatant for depressant activity in the dog. It is readily apparent that such a procedure is neither specific nor accurate.

Dorfman and Goldbaum (7), employing a chloroform extraction procedure and an ultraviolet spectrophotometric method of analysis, have determined the ability of several tissues of the rabbit to metabolize certain barbiturates. A 0.15 M phosphate buffer, PH 7.0, containing 2 per cent glucose was employed as an incubating medium. Rates of metabolism by slices, breie and homogenates were investigated. Liver slices were found to metabolize thiopental at a rate of 96 to 136 μg/gm/3 hours, breie at a rate of 20 μg/gm/3 hours and homogenates at 8 μg/gm/3 hours. Kidney slices were less active than liver slices, 72 μg/gm. being metabolized in 3 hours. Kidney breie were slightly more active than liver breie. Using liver slices and varying concentrations of thiopental or secobarbital, maximum rates were obtained at approximately 0.184 MM concentration of the barbiturates. Barbiturates, other than thiopental and seconal, which were metabolized by liver slices, included amobarbital, pentobarbital and nostal. Aprobarbital disappeared only slowly and barbital, phenobarbital and ipral not at all. In marked contrast to liver slices, kidney slices did not degrade secobarbital, amobarbital or pentobarbital. There was presented evidence for a slight metabolism of thiopental, but not secobarbital, by brain breie. Skeletal muscle minces metabolized neither of these drugs.

Kahn (8), employing 200 mg. of rat liver slices in 100 ml. of an aerated Krebs-Ringer phosphate buffer containing 6 mg. of pentobarbital, found 10 per cent (600 μg.) of the drug disappearing in 60 and 120 minutes. Alterations of calcium or potassium concentration in the buffer were without effect. Cyanide and fluoride (10^{-3} M) produced no inhibition. Addition of iodoacetamide and arsenite resulted in disappearance of pentobarbital whether the slices were surviving or heat inactivated. The action of either of these compounds was reversed 60 per cent by glutathione or cysteine. Beiler and coworkers (9) state that pentobarbital and amobarbital are metabolized by diaphragm, intestine and liver of the rat whereas thiopental disappears in the presence of the former two tissues but not in the presence of

liver. The reason for their inability to demonstrate metabolic transformation of thiopental by liver, a finding reported by other workers, is not apparent since details of method and technic are lacking.

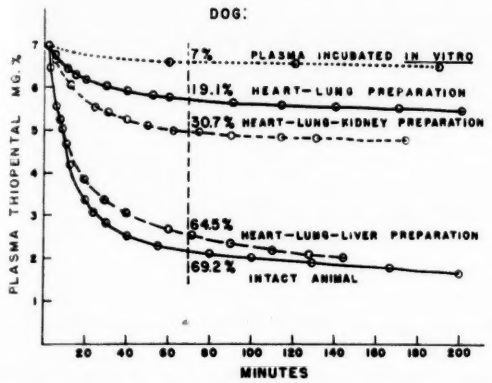

Fig. 1. Rate of disappearance of thiopental from dog plasma and from circulation of heart-lung, heart-lung-kidney, and heart-lung-liver preparations of dog. The plasma decay curve of thiopental in the intact animal is included for comparison.

Table 1. Rabbit or rat tissues which have been reported to metabolize barbiturates in vitro

Barbiturate	Tissues
Pentobarbital (Nembutal)	Liver (5, 7, 8, 9), Skeletal muscle (5, 9), Brain (5), Intestine (9), Kidney (5)
Thiopental (Pentothal)	Liver (5, 7), Kidney (5, 7), Brain (5, 7), Skeletal muscle (5, 9), Intestine (9)
Secobarbital (Seconal)	Liver (7)
------ (Nostal)	Liver (7)
Amobarbital (Amytal)	Liver (5, 7), Kidney (5), Brain (5), Skeletal muscle (5, 9), Intestine (9)
Aprobarbital (Alurate)	Liver (7)
Hexobarbital (Evipal)	Liver (6), Skeletal muscle (6), Spleen (6)

The problem of the metabolic fate of the thiobarbiturates, especially thiopental, has received a considerable amount of attention in the past few years but its metabolism, as yet, has not been completely defined (10). Attempts to approach the problem by techniques of isolation of pure metabolites from blood, urine and tissues after administration of usual pharmacological doses are rendered difficult by two factors: *1)* the possibility that there may be only minute amounts of transformation products present for detection and isolation; and *2)* the complication of innumerable interfering substances. A simpler approach to the problem might be the use of an *in vitro* technique which could be performed on a relatively large scale, and from which many interfering components, normally present in tissues but non-essential to this metabolic activity, could be removed. Once the transformation products have been characterized chemically or isolated from the system, a useful clue in the search for metabolites of thiopental *in vivo* has been supplied. The most likely tissue to employ seems to be liver in view of the general agreement on the ability of this tissue to metabolize the drug. The use of tissue slices does not appear feasible because of the technical difficulty of preparing a sufficient number of them for large scale experimentation. Minces would solve this difficulty, but they have been shown to be less active than the slice and would be objectionable from the standpoint of removal of inactive, non-essential interfering substances. The most promising preparation appears to be an homogenate which could be prepared in large quantities, would be homogeneous and which possibly could be concentrated, purified and freed of non-essential substances. The main obstacle in this approach to the problem is, as already demonstrated by Dorfman and Goldbaum (7), the lack of activity of such a preparation. However, as has been pointed out innumerable times, lack of metabolic activity in an homogenized tissue preparation may often be explained on the basis of a dilution effect, movement of diffusible metabolites and cofactors away from active centers. With this in mind experiments were instituted in our laboratory in an attempt to increase the activity of a cell-free homogenized preparation of liver by fortification with various substrates and cofactors. Mueller and Miller (11) employed such a fortified preparation of liver to study the metabolism of the dimethylaminoazobenzene carcinogens and the reaction mixture which we first employed in our studies was suggested by Mueller (12). Its composition is shown in table 2.

Stoppered Erlenmeyer flasks (50 ml.) containing the complete reaction mixture were shaken in a Warburg bath at 38° C. for 3 hours. Oxygen, saturated with water at bath temperature, was passed through the flasks for 5 minutes at the beginning of the incubation period and at subsequent hourly intervals. In later experiments the reaction mixture was incubated in 20 ml. beakers in a Dubnoff metabolic incubating shaker with oxygen supplied continuously for 3 hours. Aliquots of flask contents were analyzed (after heat inactivation) for thiopental at the beginning and the end of the incubation period by a modification of the method described by Brodie *et al.* (10). Interference due to tissue blanks was negligible. As a test of specificity of the extraction procedure and analytical method a distribution study was performed on the petroleum ether extract of thiopental from the liver homogenate preparation at zero time and at the end of 3 hours incubation. Aliquots of the petroleum ether extract were equilibrated with equal volumes of buffers at various pH values. The optical density at 285 $m\mu$ (the wave length at which acid thiopental maximally absorbs) of each petroleum ether aliquot was determined after equilibration and expressed as thiopental. This concentration was then calculated as a fraction of that in the non-distributed ether extract. The results of this study indicated that the petroleum ether extraction procedure was specific for unchanged thiopental since the distribution curves of the incubated reaction mixture and the non-incubated sample, authentic thiopental, were identical.

The results of 3 typical experiments (fig. 2) demonstrate that a properly supplemented, cell-free homogenate of rat liver is capable of metabolizing thiopental. That this disappearance of drug is enzymatically catalyzed is indicated by the fact that there is no disappearance of thiopental when the drug is incubated in the absence of liver homogenate and that no drug disappears on incubation with a heat-inactivated (15 minutes in a boiling water bath) homogenate containing the usual supplements. The following results were obtained from experiments undertaken to define the conditions necessary for optimal metabolic activity of this system.

The metabolism of thiopental was enhanced by oxygenation. In a series of Warburg flasks air was replaced with nitrogen prior to incubation. No metabolism of the drug occurred under these anaerobic conditions. Similarly, the reaction was completely inhibited by the addition of 0.02 M cyanide, at which concentration there was no oxygen uptake by the system. The rate of metabolism was determined at pH values ranging from 4.4 to 9.4. Optimal metabolic activity occurred in the range 6.4 to 9.4. Activity was diminished approximately 25 per cent below this range. That the disappearance of thiopental at higher pH values was actually a result of metabolic reactions and not due to non-enzymatic chemical alteration was substantiated by the use of aqueous controls.

The effect of varying concentrations of thiopental on its rate of metabolism by this system

TABLE 2. COMPONENTS OF SYSTEM FOR IN VITRO METABOLISM OF THIOPENTAL

1.5 ml.	Buffer, KH_2PO_4, 0.1 M (pH 7.0)
1.2 ml.	KCl, 0.02 M
0.3 ml.	$MgCl_2$, 0.1 M
0.2 ml.	H_2O
0.9 ml.	Succinate (sodium salt) 0.2 M
0.6 ml.	Nicotinamide, 0.6 M
0.3 ml.	Adenosine triphosphate (sodium salt), 0.00565 M
0.5 ml.	Cytochrome *c*, 0.00026 M
0.5 ml.	Thiopental (sodium salt), 0.0048 M
3.0 ml.	Rat liver (or other tissue) homogenate, 16.7% in ice-cold, isotonic KCl containing K_2CO_3 (0.00016 M K_2CO_3 in 1.15% KCl), prepared according to the technic described by Potter and Elvehjem (1936)

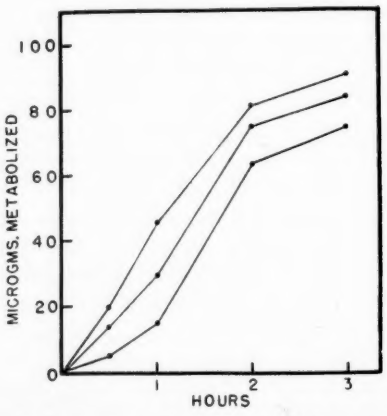

Fig. 2. IN VITRO metabolism of thiopental by a fortified, cell-free homogenate of rat liver, showing total μg. thiopental metabolized/flask at ½, 1, 2 and 3 hours of incubation in 3 typical experiments. These experiments differed from the standard procedure in that the concentration of thiopental added was 3×10^{-3} M, liver homogenate 6.7% and hexose diphosphate (0.03 M) was added in place of succinate.

was also investigated. As the amount of drug was increased to 500 μg. (0.252 mM) the total amount of drug metabolized was increased, the percentage remaining constant (fig. 3). Exceeding this level the total amount of thiopental metabolized did not increase, indicating a saturation of the metabolic system. This (0.252 mM) agrees quite well with the value (0.184 mM) calculated

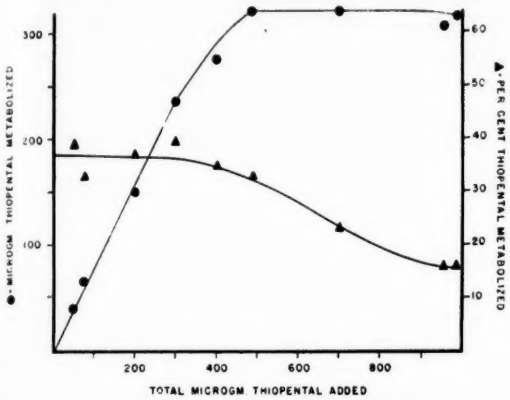

Fig. 3. Effect of increasing thiopental concentration on *in vitro* metabolism of thiopental by a fortified, cell-free homogenate of rat liver. Results of a typical experiment.

Table 3. Effect of various substrates on in vitro metabolism of thiopental by a fortified, cell-free homogenate of rat liver

Substrate (0.02 M)	Thiopental metabolized	
	Exper. 1	Exper. 2
	μg/gm/3 hr.	μg/gm/3 hr.
None	116	95
Pyruvate	136	100
Acetate	100	72
α-ketoglutarate	212	216
Succinate	240	240
Fumarate	234	203
Malate	230	211
Citrate	194	181
Hexosediphosphate	5	0

The data are from 2 representative experiments, all analyses being performed in duplicate.

by Dorfman and Goldbaum (7) for complete saturation of the metabolic system in liver slices. The proportionality between rate and drug concentration below this level could explain the observation (13) in the rat that when rate of metabolism of thiopental is calculated in terms of total dose of the drug this rate is fairly rapid during the first 6 hours, in comparison with the progressively slower rates occurring after 6 hours when the tissue levels are lower. Above a certain level of drug concentration, as noted above, metabolic rate is not increased, and consequently the percentage of total drug metabolized is actually decreased. This may be responsible partially for the relatively slow rate of metabolism observed *in vivo* in the dog by Brodie *et al.* (10) who administered relatively large doses (50–77 mg/kg.) and noted that plasma levels declined only about 50 per cent in 24 hours.

Increasing the amount of liver in the incubating medium resulted in an increase in the total amount of thiopental metabolized. Activity (μg. drug metabolized/gm. of liver) actually decreased. Since drug concentration was maintained constant this finding may be related to relative saturation of the system.

Several substrates were added to the liver homogenate and were compared with a control system, not fortified with substrate but containing all the other components. Acetate and pyruvate did little to improve the metabolism of thiopental (table 3). Krebs cycle intermediates, citrate, α-ketoglutarate, succinate, fumarate, and malate markedly enhanced the reaction. In some of the earlier experiments hexosediphosphate served as an efficient substrate but since has produced only inhibition. The explanation for this difference in activity is not apparent since the material used in both instances was from the same sample.

In control experiments the system was fortified with substrate (α-ketoglutarate or succinate) and the cofactors, cytochrome *c*, adenosine triphosphate (ATP), nicotinamide and diphosphopyridine nucleotide (DPN). To determine the importance of the cofactors in the system, each was omitted in turn, and an equivalent volume of distilled water was substituted. Absence of substrate or any of the cofactors, with the exception of DPN and cytochrome *c*, significantly diminished the activity (fig. 4). However, the omission of cytochrome *c* in a system not containing ATP resulted in a statistically significant decrease in thiopental metabolism. This would suggest that cytochrome *c* becomes a limiting factor only when there is a deficiency of ATP. The exclusion of nicotinamide resulted in the most striking decrease in metabolism of thiopental. That this inhibition was not due to an alteration in tonicity was confirmed by experiments in which isotonicity was maintained by substituting for nicotinamide appropriate concentrations of KCl.

Nicotinamide is known to prevent the destruction of DPN by active nucleosidases present in rat liver and brain (14). It was anticipated that nicotinamide would exert a greater protective effect if added directly to the KCl medium in which the liver was subsequently homogenized (15). Consequently the KCl-K_2CO_3 medium was prepared containing 0.04 M nicotinamide. The remainder of the nicotinamide was added later with the other supplements so that the final concentration, as in routine experiments, was 0.04 M. Under these conditions, however, the metabolism of thiopental was almost completely inhibited. No inhibition was noted when homogenization was performed in the presence of all components of the complete system.

Experiments were also performed to study the lability of the system. Liver, in the form of whole tissue or homogenate, was allowed to stand for 3 hours at 0° C., 25° C., and 38° C. prior to incubation with thiopental and supplementary components. In each case, as a control, an aliquot of liver was homogenized and incubated immediately after removal from the animal. Standing at 0° C., either in the form of whole tissue or homogenate, resulted in some cases in a slightly more active preparation, in others, in a slightly less active one. The activity of the homogenate was usually diminished about 30 per cent by standing at 25° C. and almost completely inactivated by standing at 38° C. for 3 hours.

Attempts also were made to fractionate and concentrate the activity of the whole liver homogenate. Samples of homogenate, prepared in the usual manner, were centrifuged in the cold at speeds varying from 500 to 2400 r.p.m. The activities of the fractions (supernatant and particulate) were not altered appreciably by variation in centrifugation speed between these limits. The supernatant and particulate were about 70 per cent as active as the original whole homogenate. Reconstituting these 2 fractions after centrifugation gave a preparation which possessed approximately the same activity as the control, whole homogenate. Calculation of activity on a tissue dry weight basis demonstrated the activity of the supernatant was generally within the range of that of the whole homogenate; the particulate matter was approximately 2 to 4 times as active. All attempts to prepare active, dried, powdered preparations were unsuccessful.

Tissues other than liver have been assayed for their ability to metabolize thiopental (fig. 5). Of all these tissues liver is the most active, followed by kidney, brain, skeletal muscle, cardiac muscle and jejunum in that order. The activity of renal tissue is in agreement with the findings of Dorfman and Goldbaum (7), and the relatively less important role of the kidneys in the metabolism of this drug *in vivo* in the rat is borne out by recent experiments in our laboratory (13). The results with brain also support the previous

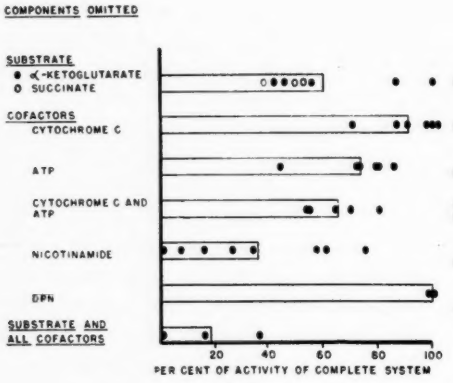

FIG. 4

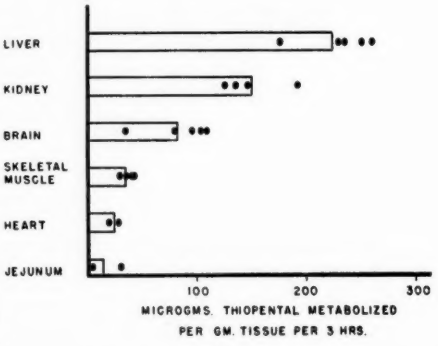

FIG. 5

Fig. 4. EFFECT of omission of substrate and cofactors on *in vitro* metabolism of thiopental by a fortified, cell-free homogenate of rat liver. Individual experiments are represented by the filled circles. All mean changes, except in those experiments involving omission of DPN or cytochrome *c*, are statistically significant, *t* values for mean differences resulting from omission of various components being: substrate, 5.19; cytochrome *c*, 1.82; ATP, 5.02; ATP and cytochrome *c*, 7.00; nicotinamide, 6.81; substrate and cofactors, 8.05.

Fig. 5. IN VITRO metabolism of thiopental by fortified, cell-free homogenates of various tissues of the rat. Individual experiments are represented by filled circles.

findings of Dorfman and Goldbaum (7) and the ability of cardiac muscle to metabolize the drug slowly is in harmony with the observations in the dog heart-lung preparation.

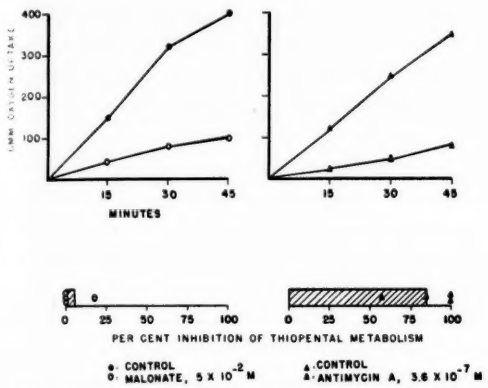

Fig. 6. EFFECT of malonate and antimycin A on *in vitro* metabolism of thiopental and oxygen uptake by a fortified, cell-free preparation of rat liver. Results of individual experiments in the metabolism studies are indicated by circles or triangles; the oxygen uptake curves are those of a typical experiment.

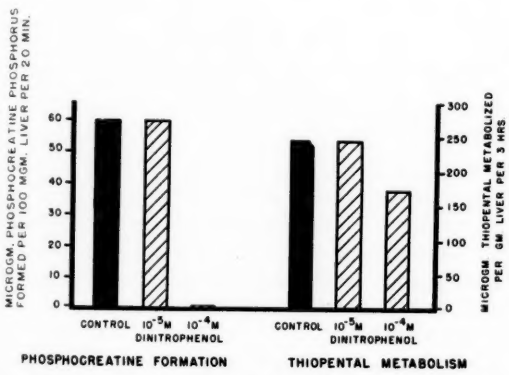
MICROGM. PHOSPHOCREATINE PHOSPHORUS FORMED PER 100 MGM. LIVER PER 20 MIN.
MICROGM THIOPENTAL METABOLIZED PER GM LIVER PER 3 HRS.
CONTROL
10⁻³M DINITROPHENOL
10⁻⁴M
PHOSPHOCREATINE FORMATION
THIOPENTAL METABOLISM

Fig. 7. EFFECT of 2,4-dinitrophenol on *in vitro* formation of phosphocreatine and metabolism of thiopental by a fortified, cell-free homogenate of rat liver. Each bar represents the results of a typical experiment. In addition to the components listed in table 1, creatine (90 mg.) and sodium fluoride (0.013 M, final conc.) were added, thus making the system suitable for phosphorylation of creatine (25) as well as metabolism of thiopental. Phosphocreatine phosphorus was measured as described by Potter (26).

Results of these studies suggest that the metabolism of thiopental probably is not accomplished by the direct action of an enzyme or enzymes, but rather by a series of enzymatic reactions from which sufficient energy is released to attack the compound. The evidence giving support to such an hypothesis is that while only slight activity is exhibited by the non-fortified homogenate, pronounced activation is brought about by the addition of an intermediary metabolite such as α-ketoglutarate, succinate, fumarate, malate and to a lesser degree citrate, and by the addition of the cofactors, ATP, cytochrome *c*, and nicotinamide. The effect of these intermediary metabolites suggests that the system responsible for the transformation of thiopental is in some manner dependent upon the Krebs tricarboxylic acid cycle.

Employing succinate as a substrate, succinoxidase inhibitors were added in an attempt to suppress succinate oxidation, and hence thiopental metabolism. Ahmad *et al.* (16) and Potter and Reif (17) have shown that Antimycin A is a potent inhibitor of succinate oxidation *in vitro*. This substance was found to inhibit oxygen uptake of the system approximately 70 per cent and to produce a high degree of inhibition of thiopental metabolism (fig. 6). The addition of malonate, however, in concentrations which depressed the oxygen uptake of the system to an equal extent, was without effect on the metabolism of the drug. Malonate has been shown to inhibit the succinoxidase system by virtue of its competition with succinate for the enzyme, succinic dehydrogenase. Antimycin A, on the other hand, has no effect on succinic dehydrogenase (17); the site of action is currently indicated at the level of the Slater factor (17). If energy from the metabolism of succinate can be utilized for the metabolic transformation of thiopental, then inhibition of succinate metabolism, either at the dehydrogenase level (malonate) or at the level of the Slater factor (Antimycin) should effectively depress the metabolism of the drug. Since malonate did not depress thiopental metabolism, an action of malonate, other than succinic dehydrogenase inhibition, was intimated. When succinate was replaced by malonate, the rate of metabolism of thiopental was usually as great as with succinate. This suggests that malonate possibly is capable of being metabolized and the energy from its metabolism utilized for the metabolic transformation of thiopental. If this is true, then it seems unlikely that the energy-yielding metabolism of malonate is aerobic, since addition of malonate to the system, in the presence of succinate, reduced oxygen uptake 70 per cent or more. In contrast, metabolism of the drug in the system containing succinate or other substrate was strictly aerobic, i.e., the metabolism of thio-

pental was depressed by omission of the oxygenation procedure and completely inhibited by lack of oxygen (nitrogen atmosphere or addition of cyanide).

Reports have appeared in the literature indicating that malonate may be metabolized. It has been shown to increase the oxygen uptake of sea urchin eggs (18), it is utilized by certain molds and bacteria (19, 20) and has been isolated from cultures of such organisms (21, 22), presumably being present as a metabolite. It has been suggested as a product of the metabolism of oxaloacetate by a pig heart preparation (23) and a recent publication by Lifson and Stolen (24) reports studies on the metabolism of C^{13}-labeled malonic acid in the intact mouse from which 20 to 30 per cent of the labeled carbon was recovered in the respiratory carbon dioxide. These investigators suggest the initial step in the metabolism of malonate is decarboxylation to acetate and that further reactions of malonate would then be similar to those of acetate, except as the presence of malonate itself has influenced metabolism. Only speculation can be made as to the role of malonate in the metabolism of thiopental. Perhaps malonate is capable of serving as a substrate (yielding energy) under conditions of depletion of normal substrate, thus explaining the increase in thiopental metabolism in the presence of malonate and the absence of succinate or other added substrate. The effects of malonate in the presence of succinate are more difficult to explain. Malonate, by virtue of its inhibition of succinate metabolism (functionally depleting the substrate) may be forced into the role of substrate. This hypothesis, it seems, would be tenable only if there were sufficient malonate present to serve as both inhibitor and substrate.

That phosphate-bond energy is needed for the metabolism of thiopental by the enzyme systems in this tissue homogenate is suggested by the fact that omission of ATP from the reaction mixture results in a decrease in the rate of metabolic alteration of the drug. The fact that 2,4-dinitrophenol, in concentrations which completely inhibited phosphocreatine synthesis in this system, suppressed thiopental metabolism only 30 per cent (fig. 7). might indicate that a source of energy other than that found in energy-rich phosphate bond compounds is available for this process.

Although these investigations have not included studies on the actual fate of thiopental, it is anticipated that this approach to the problem may prove useful for such endeavors.

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RELATIONSHIPS BETWEEN CLINICAL EFFECTS OF BARBITURATES AND THEIR NEUROPHYSIOLOGICAL MECHANISMS OF ACTION

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MUCH OF THE literature on neurophysiological mechanisms of barbiturate actions has been reviewed recently (1, 2). However, to the knowledge of this author, no general treatment of the problems involved in correlating such data with the clinical effects of barbiturates has been published. Of necessity, only tentative conclusions can be drawn from such an analysis, yet periodic evaluations of 'laboratory' data from the 'clinical' point of view are profitable, since they may influence to some degree the direction of future research.

In this report, an attempt will be made to make explicit the 'operational' meaning of terms used in the description of data. This requires that 'phenomena' be described in terms of actual procedures which the observer carries out, including an account of the organism studied, its history and environment, the instruments employed, the experimental arrangements and the method of handling the data—in short, the 'operation' involved. The necessity for defining 'phenomena' in this way becomes apparent when we consider that the effects of barbiturates vary greatly at different dose levels, and that even when the latter are known, changes in the experimental situation may alter the effects of the drug markedly. Thus, after intravenous injection of 0.25 to 1.0 gm. amobarbital, a subject may fall asleep if he lies in bed undisturbed, yet he may be awake and voluble if interviewed by a psychiatrist; or, he may exhibit ataxia on attempting to walk back to his bed, but he may 'sober up' promptly when instructed to pose for a motion picture demonstration of ataxia. Therefore, it is hazardous to infer mechanisms of barbiturate actions without taking into account both the dose level, and the 'state of the organism' at the time observations are made. Since the latter may vary considerably in different experiments, it is evident that correlations between different phenomena can be made with confidence only when they are observed to occur in the same experiment, or in a series of experiments in which the effects of uncontrolled variables have been cancelled out. Often, however, this is not possible. Thus, when some of the more recently developed electro-physiological technics are used (e.g., evocation of cortical potentials by stimulation of peripheral sensory nerves), only reflex activity may be observed concomitantly, since relatively deep anesthesia is required for elimination of background cortical electrical potential changes. Therefore, correlations may be made only between the effects of barbiturates on the electrical phenomena under observation, and the depth of anesthesia, as judged by changes in other phsyiologic variables. If, on the other hand, the effects of barbiturates are observed by the use of some other type of procedure (e.g., studies on adaptive goal-directed behavior), electrophysiological studies of the sort just described cannot be carried out concurrently. In the face of this operational dilemma, attempts are often made to 'explain' barbiturate effects by analogy with the effects of excitation or destruction of various discrete portions of the nervous system. Such formulations of mechanisms of drug action may prove to be quite erroneous, since agents like barbiturates have widespread actions in the nervous system, and diverse mechanisms may produce similar patterns of change. Other potential sources of error involve assumptions that are often made tacitly—that various concurrent responses to a given stimulus are equivalent to each other, or that one response is the cause of another. The fallacies inherent in such assumptions have been analyzed in some detail elsewhere (3, 4). They will become more apparent when a number of phenomena of interest to the clinician are considered in relation to the neurophysiological mechanisms of barbiturate actions.

ANALGESIA

Operational analysis of what is meant by 'analgesia' in the clinical sense reveals that this term refers to a wide variety of phenomena. Ordinarily, the observer asks the subject, who already has reported that 'it hurts,' whether an agent administered previously has effected any change in the pain; the 'analgetic' potency of the compound in question is assayed in terms of the subject's verbal report, or his non-verbal behavior. This procedure can be quantified adequately by standardization of environment, selection of patients and proper statistical analysis of the data (5). However, 'it hurts' or 'it hurts less' may mean one thing or another, and the verbal report may or may not be compatible with the subject's non-verbal behavior, thus making it necessary to use some arbitrary measure of 'analgesia.' Furthermore, such a measure must also take into account the presence or absence of undesirable 'side-effects,' such as confusion or neuromuscular dysfunction, for there are 'good' and 'poor' analgesics, from the clinical point of view. Nevertheless, this procedure is doubtless the most reliable direct method of evaluating analgesic potencies of drugs for clinical purposes, and with it, Keats and Beecher (5) have demonstrated that 60 or 90 mg. pentobarbital (per 70 kg. body weight) are effective in relieving post-operative pain in about 50 per cent of patients, under certain specified conditions. These authors have pointed out that psychologically the mechanism of pain relief from barbiturates appears to be a reduction of concern about pain, rather than a reduction in the intensity of perceived pain, and they have drawn analogies between this mechanism and that of relief of pain by frontal lobotomy. However, as noted above, such an analogy cannot establish a locus of action of barbiturates. Neither can one ascribe with confidence the analgesic action of barbiturates to their depressant effects on internuncial neuron activity, since procedures which demonstrate such actions preclude the concurrent measurement of analgesia as defined above. Nor would a cause-effect relationship between these two phenomena be established if it were possible to demonstrate their concurrence.

It is probably more than a coincidence that opiates (2) and barbiturates (6, 7) both of which have analgesic properties, also depress internuncial activity. Yet clinical experience has shown that mephenesin, which has powerful depressant effects on internuncial activity in animals (8), is of very limited value as an analgesic in man, at least in doses that may be administered safely. Evidence has been presented recently which indicates that the relief of pain by mephenesin, when it does occur, is effected through a peripheral mechanism of action (9). These discrepancies make it likely that there is no one-to-one relationship between analgesic effect of drugs like opiates, barbiturates and mephenesin and any particular neurophysiological mechanism of action. Rather, a correspondence may be drawn between the *patterns* of clinical effects and *patterns* of neurophysiological actions of such drugs. Thus, morphine relieves pain clinically in doses which have few undesirable 'side-effects;' it also depresses internuncial neuron activity in a highly selective manner at various levels in the central nervous system. To produce comparable degrees of pain relief by barbiturates or mephenesin, doses of these drugs must be employed which produce more definite evidence of drowsiness, confusion or muscular weakness than morphine does; neurophysiologically, the effects of barbiturates and mephenesin are more diffuse than those of morphine (2).

EUPHORIA, DYSPHORIA AND THE 'NARCOANALYTIC EFFECT'

'Euphoria' is a term which has an even greater variety of meanings than 'analgesia.' It is commonly defined as a sense of unusual well-being. However, what constitutes 'well-being' for one person may differ radically from that in another, or for the same person at different times in different situations. Thus, after morphine, a post-addict may vomit and appear pale and faint, yet he reports that he feels 'unusually well.' In such subjects, the euphoric state seems to be related to reduction of fear of pain, hunger and sexual urges (10), rather than to impairment of contact with the external environment. In the same type of individual, a sense of 'unusual well being' is not experienced after barbiturates except in doses which produce evidence of gross intoxication. However, euphoria then appears to be associated with loss of self-control, 'acting-out' of hostility and sexual aggressiveness and impairment of the sensorium, with 'anosognosia' or denial of illness. It appears to be likely that in non-addicts, 'euphoria' also describes phenomena which differ widely in individuals and in the same person at different times. Likewise, 'dysphoria' may occur after barbiturates in particular in-

dividuals or in particular situations. Thus, a post-addict who has been receiving intoxicating doses of barbiturates may be 'euphoric' if undisturbed, but may become agitated or 'excited' in the psychological sense of the term, if a further increase in dosage of the drug is denied. Similar 'excitant' effects of barbiturates may also be observed in non-addicts. Possibly in some this may be due to biochemical idiosyncrasies, particularly if such reactions occur consistently in widely differing situations. However, in most, 'excitant' effects of barbiturates appear to be related to the special circumstances which prevail at the time the drug is administered. These considerations apply also to the 'narcoanalytic' effect of barbiturates. The intravenous route of administration is more effective than others in producing this state, and the relationship of the subject to the observer is also an important determining factor.

Euphoria, dysphoria and the narcoanalytic effect appear to be related to each other in at least one respect, namely, the presence of some degree of demonstrable impairment of the sensorium. Obviously, this cannot be related to any particular neurophysiologic mechanism of action of barbiturates, since impairment of the sensorium occurs clinically in a wide variety of neurologic disorders. Again, we can only show a correspondence between such clinical effects and the changes noted in patients with diffuse organic brain disease (11), or the widespread depressant effects of barbiturates in the central nervous system. Certainly, the terms 'release' and 'excitement,' which are often used in describing some clinical actions of barbiturates cannot be equivalent in meaning to the corresponding neurophysiological terms, since the operations by which either of these two classes of phenomena can be demonstrated, preclude those which are necessary for the demonstration of the other.

NEUROMUSCULAR INCOORDINATION

In doses sufficient to produce evidence of gross impairment of the sensorium, barbiturates also produce ataxia, dyssynergia and nystagmus. These changes are very similar to those exhibited by patients with cerebellar disease and, by analogy, the effects of barbiturates may be ascribed tentatively to 'depression' of the cerebellar and vestibular systems. However, there appears to be no direct evidence that this is the case. Seemingly, it would be very difficult to study concurrently patterns of 'voluntary' muscular movement and the effects of barbiturates on these systems in isolation.

ANTICONVULSANT ACTIONS

Epilepsy in man is multifactorial in origin, but the muscular activity associated with seizures must involve the repetitive discharge of motoneurons. This particular aspect of epilepsy has been studied extensively and the effects of barbiturates and other anticonvulsants thereon have been reviewed in detail by Toman (12). Increase in threshold of nerve for responses to electrical and chemical stimuli, coupled with prolongation of recovery time, appear to account for the anticonvulsant actions of such drugs.

SEDATION, SLEEP AND ANESTHESIA

These clinical phenomena appear to be closely related, since under favorable circumstances they may succeed each other with increasing dose levels of barbiturates. Operationally, they are defined in terms of the degree of responsiveness, verbal, behavioral or reflex, which an individual exhibits when subjected to a variety of stimuli. Technically, it is possible to study sleep and anesthesia in animals, and great advances have been made recently in the elucidation of neurophysiological correlates of these phenomena. For convenience, we may consider separately the data on evoked thalamic and cortical potentials, spontaneous electrical activity of the cortex, and spinal reflexes.

Marshall (13) demonstrated that in cats under pentobarbital anesthesia, tactile or electrical stimuli applied to peripheral sensory nerves evoked measurable electrical responses in the tegmentum, thalamus, internal capsule and cerebral cortex. During deep anesthesia, the absolutely unresponsive time for the reaction rostral to the ventrolateral nucleus pars externa of the thalamus was found to be 25 to 50 msec., whereas the absolutely unresponsive time caudal to the thalamus was too brief to be measured. Jarcho (14) demonstrated that deepening pentobarbital anesthesia is also associated with an increase in absolute and relative recovery time at the cortical level, though part of this effect of barbiturates may be due to depressant effects in the thalamus. Forbes and his co-workers (15) showed that evoked 'primary' responses, mediated topically to the cortex through thalamic relay nuclei, were more resistant to the depressant effects of pentobarbital but evoked 'secondary' responses, mediated more diffusely to the cortex via extra-

thalamic pathways, were markedly affected by the drug. This was indicated by progressive increase in total refractory period at the cortical level, from 0.2 to over 1 sec., as anesthesia deepened. The depth of anesthesia was estimated in terms of a 'functional mean' of various measurements, including respiration, blood pressure and body temperature. These data indicate that with deepening barbiturate anesthesia there is a progressive decrease in the number of electrical changes that sensory impulses can produce at the cortical level in a given unit of time.

Bremer (16) demonstrated that after transection of the midbrain the EEG of unanesthetized cats assumes the typical 'burst' pattern of natural sleep, or after induction of sleep by pentobarbital; this investigator attributed such EEG changes to 'deafferentation' of the cerebral cortex. However, Lindsley, Bowden and Magoun (17) and Morruzzi and Magoun (18) showed that this effect was due, not to interruption of sensory impulses enroute to the cerebral cortex via thalamic relay nuclei, but to inactivation of a basal diencephalic reticular 'desynchronizing' mechanism, which probably inhibits an opposed medial and intralaminar thalamic 'synchronizing' mechanism. The latter appears to be related to a 'diffuse projection system' in the thalamus, which is not directly involved in the transmission of sensory impulses but which regulates the background electrical activity on which sensory impulses must act at the cortical level (19). It would appear, therefore, that barbiturates alter the EEG primarily through a depressant action on the basal diencephalic reticular 'desynchronizing' mechanism. The studies referred to also indicated that a close relationship exists between this mechanism and those involved in sleep and wakefulness. However, it is possible that the two are not identical, since Magoun (20) has found that after the production of large lesions in the hypothalamus the EEG may be desynchronized by sensory stimulation while the animal remains quite somnolent. A different relationship between the EEG and hypnotic effects of barbiturates has been suggested by Swank and Watson (21). Their evidence (rather indirect) indicated that amobarbital depresses the electrical activity of smaller cortical neurons selectively, and they attributed the unconscious state which this drug may produce to 'imbalance' of cortical functions.

With adequate doses of barbiturates, all spinal reflexes may be depressed markedly (22, 23, 24). However, selective depression of interneurons at the spinal level may occur after smaller doses (25). The mechanism of depression of the monosynaptic reflex arc has been elucidated in electrophysiological terms by Brooks and Eccles (26), who found that with deepening pentobarbital anesthesia, motoneurons could be fired only by progressively higher synaptic potentials which were developed in the vicinity of the motoneurons following afferent stimulation. These observations indicate that pentobarbital 'stabilizes' the electrical membrane potential of the motoneuron soma.

CONCLUSIONS

It is apparent that much more than a knowledge of neurophysiological mechanisms is required for an understanding of the genesis of clinical phenomena and the effects of barbiturates thereon. In part, this may be due to current inadequacies of our technics of observation. However, a consideration of what 'neurophysiology' means operationally indicates that neurophysiological procedures alone will never be entirely sufficient for this purpose.

The neurophysiological approach to the explanation (i.e., the prediction) of clinical phenomena may be defined as a technic which involves the making of correlations between nervous structures and functions. 'Structure' is defined in terms of various kinds of procedures—the division of the nervous system into parts on the basis of external appearance, phylogenetic relations, cytoarchitecture, afferent, efferent and internuncial systems of different sorts, nuclei, tracts, etc. 'Functions' are defined in terms of procedures involving activation or inactivation of various parts, or combinations of parts of the nervous system, and the subsequent observation of changes in activity of various sorts—electrical, muscular, visceral, secretory, behavioral, verbal, etc. It would be surprising indeed if the data obtained by these diverse procedures (and their diversity is far greater than already indicated) were wholly commensurable. That often they are not, even where they might reasonably be so, has been pointed out by Walshe (27) in a consideration of the functions of the motor cortex. The hope that 'ultimately' they will be is a view that may be called 'reductionism.' This view assumes that the functions of the whole organism can be understood as the sum of functions of its parts. Since, in the last analysis, 'parts' consist of atoms (or even smaller particles and/or waves), it is

held by some that eventually the behavior of man will be explained in terms of the concepts of nuclear physics. The fallacies inherent in this assumption have been dealt with by Sloane (28).

More consistent with the facts is the view that "... the so-called properties of the bodies revealed by our measurements are in fact primarily the reflex of our measuring methods and not concrete facts *in rebus naturae*" (27). Furthermore, the organism, its environment and the observer (including his instruments) constitute a unit, in terms of which 'properties' must be expressed. In reality, therefore, any given experimental procedure yields data that are unique. Nevertheless, for the purpose of ordering our data, we group experimental procedures into various loosely defined categories ('psychological,' 'physiological,' 'biochemical,' etc.) and we construct concepts of 'functions' relative to each category which enable us to summarize the data acquired by experimental procedures which are more or less alike. It is evident, however, that since actual carrying out of one type of procedure ordinarily precludes the concurrent carrying out of procedures of a different category, cause-effect relationships, or even perfect correspondence between 'functions' in different categories can rarely if ever be demonstrated. Also, since every experimental procedure entails some alteration in the organism, its environment or both, the range of phenomena which can be described in terms of any given procedure is definitely limited.

It is therefore necessary to redefine precisely what we mean by 'stimuli,' 'responses' and 'mechanisms.' A 'stimulus' is a change in experimental arrangements (delivery of an electric shock, injection of a chemical, ringing a bell, exhibiting a word, etc.) which is followed by changes involving the organism under observation, called 'responses.'

Changes in experimental arrangements, however, often are not limited to those over which the observer has control. Therefore, a 'stimulus' can be defined operationally only in terms of a particular response which is evoked by such changes in experimental arrangements as are known to the observer. Thus, a stimulus with known fixed physical properties may be 'subnormal,' 'threshold' or 'supramaximal,' depending on particular experimental conditions which prevail when it is applied to the organism, and the history of the latter.

Changes involving the organism are known to us only in terms of the changes they produce in our instruments of observation, namely, our recording devices and/or the perceptive apparatus of the observer. Serial 'responses' following stimulation can therefore be described accurately, with respect to time sequence, only with reference to the changes which are produced in a given instrument of observation, which may reflect changes in verbal or non-verbal behavior, or changes of mechanical, electrical or chemical nature.

'Mechanisms' are operational constructs by use of which we relate serial changes in a particular instrument of observation to stimuli, in terms of a given type of experimental procedure. Hence, there is not likely to be a demonstrable cause-effect relationship or a one-to-one correspondence between 'psychological,' 'physiological' and 'biochemical' mechanisms, and one should recognize that words like 'stimulation,' 'depression,' 'threshold,' 'reaction,' 'inhibition' and 'release' mean quite different things when used in connection with such different procedural categories. However, 'mechanisms' in different categories may be interrelated, since, generally, it is the undivided organism that is responding to stimuli. Consequently, any particular change in a given instrument of observation may be produced in part by many different mechanisms.

It follows, therefore, that the effects of a given stimulus upon the organism can be predicted with a reasonable degree of probability only by the use of multiple technics of observation and experimental procedures, and that these will never be reduced to some all-embracing 'mechanism,' physical, chemical or other. Highly quantified studies on the smallest anatomically defined biological units are necessary and useful, since they enable us to control variables, but they are to be regarded as studies on simplified but artificial models of the nervous system. Mechanisms which are inferred from data obtained by the use of such models must be reformulated repeatedly in order to include the results of studies with similar procedures on other more complex models, and a definite limit is set for this process by the technical requirements of neurophysiological procedures. This view is not incompatible with philosophic monism (3). Rather, it emphasizes a necessary pluralism of operational frames of reference and the need for some degree of flexibility in formulating concepts of 'properties,' 'functions' and 'mechanisms.'

From the standpoint of clinical neuropharmacology, another conclusion appears to be war-

ranted. Many drugs, like barbiturates, are ubiquitous in their sites of action in the central nervous system. Therefore, it is hazardous to 'explain' a given clinical action of such drugs by analogy with the effects of localized activation or inactivation of specific nervous structures. Also, such drugs affect various mechanisms simultaneously, a situation which is ordinarily avoided in neurophysiological experimentation. Hence, it may be more prudent to limit 'explanations' of the actions of such drugs to the description of *patterns* of action in terms of the largest possible variety of 'mechanisms,' psychological, physiological and biochemical, which can be demonstrated. Such an approach may make it possible to classify drugs in terms which are highly specific. The *pattern* of action of a new drug may thus be compared with the patterns of action of known drugs and perhaps predictions concerning its clinical usefulness can be made on this basis.

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ENZYMATIC ASPECTS OF BARBITURATE ACTION

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THE BULK of our present knowledge pertaining to the interaction of barbiturates and enzymes stems from the classical observations by Quastel and his coworkers (1–5) that narcotics of various types, among them the barbiturates, are capable of inhibiting the respiration of brain and other tissues *in vitro*. The work of the Quastel school has been confirmed many times by subsequent investigators (6–22). There seems little doubt therefore, that one of the possible ways in which barbiturates produce their pharmacological effects is by an inhibition of the oxidative processes of the cell. Whether or not this is their chief site of action is questionable. I should like to review the evidence pro and con for this hypothesis, to point out some of the other possibilities and the evidence therefor, and to indicate some avenues of investigation which might lead to a more satisfactory explanation of the mechanism of barbiturate action on an enzymatic basis.

There is little evidence of interaction of the barbiturates with enzymes other than those which are directly or indirectly concerned with the energy-transforming mechanisms of the cell. This discussion will be confined almost entirely to such enzyme systems.

Figure 1 is a block diagram indicating in descriptive terms the divisions into which the energy transformations of the cells may be classified. It is my hope that this diagram will serve to orient and clarify the subsequent presentation.

The first energetic transformation which the cell carries out is the production of energy by the oxidation of carbohydrate or other substances (the brain is presumed to utilize chiefly carbohydrate). It is to this stage that most of the data on barbiturate-enzyme interactions apply.

Table 1 summarizes the data of a number of investigators (1–22) on the effects of the barbiturates on the overall oxidation of glucose and pyruvate by slices or homogenates of brain tissue. There is a certain amount of variation from investigator to investigator in the concentration of barbiturate required to produce 50 per cent inhibition (I_{50}) of oxidations, so that the data listed represent something of a compromise, but they are surely of the right order of magnitude and will serve to illustrate the points. It may be seen that concentrations of barbiturate are found which will give 50 per cent inhibition of oxidations *in vitro* and I am sure that no one would quarrel with the assumption that a decrease in the oxidative capacity of a tissue by 50 per cent could have profound effects on the ability of that tissue to carry out functional tasks.

There has been much discussion as to the exact site in the oxidative systems of the cell at which narcotics act to produce the observed inhibitions of oxygen consumption. There is evidence of an indirect nature that cytochrome *c* reductase (7, 8) is the sensitive site. There is also a contention that the pyruvic oxidase system is most sensitive and that it is the dehydrogenase moiety which is inhibited (17). However, in

Fig. 1. SCHEMATIC representation of energy transforming systems.

view of the recent developments on the complexity of the cytochrome chain (23) this whole question is now open for reinvestigation. Since I wish to adopt the point of view that inhibition of respiration *in vitro* is not a sufficient explanation for *in vivo* effects of the barbiturates it does not seem appropriate to discuss the above question here, although it may be possible to relate some of the findings to later arguments.

The chief difficulty with applying the type of data given in table 1 to the explanation of *in vivo* effects is illustrated in the last column. This column gives the ratio of the I_{50} concentration to the concentration calculated to exist in the central nervous system during anesthesia, assuming a uniform distribution of intravenously administered drug throughout the body (8, 24–34). We see that in most cases at least 4 times the anesthetic concentration and in some cases as much as 30 times the anesthetic concentration is required to produce 50 per cent inhibition of oxidation *in vitro*. Only one barbiturate, hexethal (not shown in the table), has been reported to yield an I_{50} the same as the AD_{50} (6). One ex-

planation for this disparity of concentrations has been that the barbiturates are concentrated preferentially in the brain. This is apparently the case only with pentobarbital which perhaps is concentrated 1½ times (33, 34, 35) and with amobarbital which is reported to concentrate 4-fold (28). If we accept the possibility of a 4-fold concentration of amobarbital in the brain this brings the ratio, I_{50}/AD_{50}, close to one for only 2 of the barbiturates, namely, hexethal and amobarbital. All others give ratios of from 4 to 30.

Another rationalization which has been advanced for this concentration difference is that there is a unique sensitivity of the energy-producing systems of a critical region in the central nervous system. Evidence to support this contention is not available and the data of Wilkins *et al.* (11, 16, 20) indicate that a given concentration of pentobarbital produces essentially equal depressions of oxygen uptake of slices from the caudate nucleus, hypothalamus and cerebral cortex.

Still another possible rationalization is that some critical site of the central nervous system has a much higher energy requirement than normal and that it is operating close to capacity so that the small inhibition of oxidation which can be expected to occur at anesthetic concentrations would significantly affect the function of that site. In view of experience with most cells, however, it is found that there is usually a certain reserve capacity in the energy production system and that it is exceeded only under conditions of extreme stress. There is no doubt that various parts of the central nervous system, various sizes of cells, and various sizes of fibers show different sensitivities to barbiturates *in vivo*, but this is not necessarily related to the sensitivity of their oxidative mechanisms.

On the other hand there are *in vivo* observations which are not explained by simple inhibition of oxidative processes:

1) Himwich *et al.* (36) have shown that the cerebral metabolic rate is not depressed until deeper levels of anesthesia are reached, and much functional depression can be demonstrated before a depression of the cerebral metabolic rate.

2) Larrabee *et al.* (37) report that conduction through ganglia can be depressed by concentrations which do not affect oxygen consumption measured simultaneously.

3) Low concentrations of drug can cause stimulation of the intact nervous system either by inhibition of regulative centers or by direct action.

4) Even though succinate oxidation is not depressed *in vitro* by barbiturates, succinate exerts no analeptic effect on barbiturate anesthesia (32, 38).

5) Barbiturates lower phosphocreatine and raise inorganic phosphate levels *in vitro* (12, 13, 14), an effect which would be expected if oxidation were inhibited, but just the opposite effect is observed *in vivo* (14, 39). The same thing may be said for lactic acid.

6) It has been shown by Dr. McIlwain, who has kindly given me permission to quote his results prior to publication, that 1 to 5 $\times 10^{-4}$ M phenobarbital (concentrations below or equal to the anesthetic concentration, table 1) inhibits

TABLE 1. EFFECT OF BARBITURATES ON BRAIN TISSUE OXIDATIONS IN VITRO

BARBITURATE	TISSUE PREPARATION	SUBSTRATE	I_{50}[1]	AD_{50}[2]	I_{50}/AD_{50}
			Molarity	*Moles/kg.*	
Amobarbital	Slices	Glucose	7.5×10^{-4}	2.5×10^{-4}	3.0
	Homogenates	Pyruvate	5.2		2.1
Pentobarbital	Slices	Glucose	8.5	2.0	4.3
	Homogenates	Glucose	9.0		4.5
		Pyruvate	5.0		2.5
Thiopental	Slices	Glucose	28.5	0.8	35.6
Phenobarbital	Slices	Glucose	40.0	6.0	6.7
	Homogenates	Pyruvate	36.0		6.0
Barbital	Slices	Glucose	480.0	15.0	32.0
	Homogenates	Pyruvate	270.0		18.0

[1] Concentration of barbiturate required to inhibit oxygen uptake 50%. Approximated from data given in references (1–22).

[2] Anesthetic $dose_{50}$ calculated assuming a uniform distribution of an intravenous dose throughout the body, or from actual blood and brain levels as reported in references (8, 24–34).

the extra oxygen uptake resulting from electrical stimulation of brain slices *in vitro* without inhibiting the unstimulated rate.

Other objections may no doubt be called to mind, but the above are certainly sufficient to make the point that inhibition of oxidation *per se* is not an entirely satisfactory explanation for barbiturate action.

One explanation for some of the above effects has been the postulation that an alternative pathway for glucose oxidation exists (36) which is directly concerned with function and which might conceivably be more sensitive to barbiturate inhibition. There is evidence for an alternative pathway, but the rest of the hypothesis has not yet been put to experimental test.

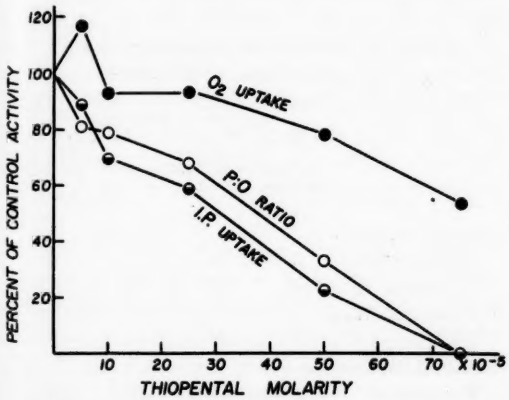

Fig. 2. EFFECT of thiopental on oxidative-phosphorylation in rat liver mitochondrial preparations (43).

In figure 1 it can be seen that the phase which has been discussed is only the first operation which the cell performs in the energy transformation scheme. The next step is the transfer of the energy so produced to a 'storage' system upon which the cell may draw to perform its various tasks. I have not said much about the details of the energy production systems because current concepts of the steps through which glucose is degraded are familiar. It is generally accepted that the storage system of the cell is in the high energy phosphate bonds of adenosinetriphosphate and phosphocreatine, with the first of these presumably being the immediately available source of energy for function and phosphocreatine acting to maintain adenosine-triphosphate levels. However, figure 1 indicates that there is a step between 'production' and 'storage' which is termed 'coupling mechanisms.' The exact details of these mechanisms are, for the most part, not as yet known[1]. We know that electrons are transferred in a stepwise fashion through the cytochrome chain and it is thought that each step corresponds to the uptake of 1 mole of inorganic phosphate into a high energy bond. However, even though the intimate details of the chemical reactions are still obscure, we know that during the oxidation of carbohydrate (and some other substances) there is a concomitant appearance of high energy phosphate bonds in the form of adenosine-triphosphate. For the most part, the enzymes which catalyze this transfer of phosphate from inorganic to organic forms are extremely labile and require specialized techniques to maintain them at their maximum efficiency in any cellular preparation suitable for *in vitro* study.

We know that certain chemicals can interfere with this coupling mechanism and allow oxidation to wastefully proceed in the sense that no high energy bond formation accompanies it. We know, in addition, that if the cell is deprived of its ability to generate high energy phosphate its functional capacity is severely impaired.

We come then to another possible site of action for the barbiturates, namely, interference with this coupling or transferring mechanism. Such a site of action has been suggested in the past (41), but attempts to demonstrate interaction of these enzymes with narcotics were unsuccessful (9, 12, 42), probably because the systems used were not adequate. However, with the use of mitochondrial preparations such an interference by at least 4 of the barbiturates can be demonstrated, and it occurs to a significant extent at concentrations of the drug which do not give much effect on oxidation (43).

Figure 2 illustrates the effect of thiopental on this transferring mechanism. These results were obtained on a preparation of mitochondria from rat liver, but as will be seen in table 2 the same effect can be demonstrated on preparations from brain. Here we see that inhibition of the formation of high energy phosphate bonds, as indicated by the inorganic phosphate uptake, is inhibited 40 per cent by concentrations of barbiturate which yield less than 10 per cent inhibition of oxygen uptake. The uptake of oxygen is still at 50 per cent of its normal value at concentration of drug which inhibit completely the uptake of inorganic phosphate.

[1] See, however, articles by S. P. COLOWICK AND N. O. KAPLAN (40).

Table 2 shows the extension of this type of study to other barbiturates. Here it can be seen that 50 per cent inhibition of inorganic phosphate uptake is obtained with concentrations of amobarbital and pentobarbital which are only 1½ times the anesthetic concentration. If we allow the 4-fold concentration in the brain for amobarbital and the 1½-fold concentration for pentobarbital, as we did in interpreting the oxygen uptake data on slices in table 1, we have an effect which clearly must be of significance *in vivo*. With thiopental the concentration required to produce an inorganic phosphate uptake inhibition of 50 per cent is still 4 times the anesthetic concentration, but this compares with the 30 times required to give 50 per cent inhibition of oxygen uptake in slices (table 1). Further, when inorganic phosphate uptake is inhibited 50 per cent oxygen uptake in mitochondria is inhibited only 20 per cent.

Phenobarbital does not exhibit much inhibition until concentrations of 1×10^{-3} M are reached, but this is only 2 times the anesthetic concentration as compared to the 6 times required to give 50 per cent inhibition of oxygen uptake in slices (table 1).

All of these data were obtained with mitochondria respiring on pyruvic acid as the principal substrate, but it may be mentioned that the same effect can be demonstrated with alpha-ketoglutarate, glutamate, or succinate (44).

This uncoupling effect, then, demonstrates that there is at least one enzymatic site which is more sensitive to the action of barbiturates than is oxidation *per se*. It also offers a possible explanation for certain other observations, for example:

1) Westfall (10, 18, 19) has reported stimulation of oxygen uptake in brain slices at low concentrations of pentobarbital (2×10^{-5} M) and amobarbital (8×10^{-5} M). Such an effect is often observed in organized cellular systems under the influence of uncoupling agents and is explicable in terms of the postulation of Johnson (45) that the rate of oxidation is regulated by the level of inorganic phosphate and adenylic acid in a limited system such as the cell. The same explanation may be applied to the observed stimulation of aerobic glycolysis by barbiturates in brain suspensions (22).

2) McLennan and Elliott (46) have found that pentobarbital, amobarbital and phenobarbital inhibit the synthesis of acetylcholine in brain slices at concentrations of 5×10^{-4} M. This concentration is that which gives almost complete inhibition of high energy bond formation (table 2). Since acetylcholine synthesis requires high energy phosphate, an explanation for the effect is at hand in the uncoupling phenomenon. The stimulatory effect of still lower concentrations of barbiturate on the synthesis of acetylcholine (46) is still not explicable.

3) The failure of succinate as an analeptic for barbiturates (32, 38) is also explicable since the uncoupling effect is demonstrable on the uptake of inorganic phosphate associated with the oxidation of succinate (44) as well as other substrates.

4) Perhaps the most salient point, however, is that such an uncoupling effect furnishes a possible explanation for the fact that function can be depressed without depression of oxygen uptake. Unfortunately, the publications of Larrabee *et al.* (37) are not detailed enough to permit a comparison of the concentrations required to depress conduction through ganglia and those which we find *in vitro* to inhibit phosphorylation.

TABLE 2. EFFECT OF BARBITURATES ON OXIDATIVE-PHOSPHORYLATION[1]

	PERCENTAGE INHIBITION AT 2.5×10^{-4} M.		PERCENTAGE INHIBITION AT 5.0×10^{-4} M.	
	OXYGEN UPTAKE	INORGANIC PHOSPHATE UPTAKE	OXYGEN UPTAKE	INORGANIC PHOSPHATE UPTAKE
Liver Mitochondria				
Pentobarbital.. 5	16		61	92
Amobarbital... 10	30		64	92
Phenobarbital.			31	36
Thiopental.... 7	41		32	78
Brain Mitochondria				
Thiopental.... 17	40		29	70

[1] From Brody and Bain (43).

The postulation of barbiturates as uncoupling agents is not without its anomalies. It is difficult, for example, to understand why barbiturate administration does not lead to a rise in inorganic phosphate in the blood (47) or in the brain (39). Then, too, why don't barbiturates raise body temperature in the same way in which the classical uncoupling agent, dinitrophenol, does? For that matter, why isn't dinitrophenol itself an anesthetic agent[2]? These and other questions cannot be answered in an unequivocal manner at present.

[2] Since the presentation of this paper we have been able to show that barbiturate anesthesia is potentiated by dinitrophenol and that the latter drug is itself a depressant.

Returning again to figure 1, it may be said that there is no known action of the barbiturates on 'storage' *per se*. As a matter of fact the barbiturates might possibly aid the cell in this respect by inhibiting phosphatases which would tend to break down the high energy phosphate compounds. On the other hand we have found that phenobarbital, at least, has no inhibitory action on adenosinetriphosphatase from brain (44).

The step between 'storage' and 'utilization' is indicated as being made up of another set of 'coupling mechanisms.' Our knowledge as to the nature of these reactions, particularly in the case of nervous tissue, is very meager indeed. It can be said, without serious extrapolation, that adenosinetriphosphate is certainly intimately concerned. It is not illogical to suggest that if the barbiturates have properties which allow them to interact with the transferring enzymes which mediate the synthesis of adenosinetriphosphate, they may also be able to interact with the enzymes which mediate the breakdown of adenosinetriphosphate and the associated performance of function.

It is suggested, then, that these last stages of energy transformation are a fertile field for investigation. Such investigations may lead us to the discovery of an enzymatic site which is still more sensitive to the action of barbiturates, and for that matter, other drugs.

We may speculate that nervous function is related to membrane permeability. It is known that adenosinetriphosphate can change the properties of the specialized proteins of muscle (48). Perhaps it can also change the properties of the proteins of the nerve cell membrane.

We may speculate that nervous function is related to the maintenance of ionic gradients (49). Terner *et al.* (50) have shown that brain slices maintain potassium ion concentration gradients at the expense of the oxidation of glucose and glutamate, presumably with the mediation of adenosinetriphosphate since uncoupling agents inhibit this ionic concentration process in at least one other tissue (51).

We may speculate with Nachmansohn and others (52–54) that acetylcholine is somehow concerned with nervous function and that its synthesis represents a 'recharging' mechanism. In this regard we have the observations that the barbiturates interfere with the synthesis of acetylcholine (46) and it should be pointed out that such an interference can be explained not only by a block of the synthesis of adenosinetriphosphate as was postulated above, but also by interference with adenosinetriphosphate's participation in the reactions leading to the synthesis of acetylcholine.

Whatever the actual, detailed explanation for nervous function, be it based on any of the above, alone or in combination, or be it some set of reactions unknown or unsuspected at present, it is my strong belief that ultimately we will know the answers. When we do, we will be able to see the various *in vitro* effects of barbiturates in their proper perspective and will be able to decide which one of these effects or which combination of effects is a satisfactory explanation for *in vivo* observations.

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AMERICAN INSTITUTE OF NUTRITION

Symposium on Utilization of Protein[1]

Chairman: A. H. SMITH

PROTEIN NUTRITION IN SURGICAL PATIENTS

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IT IS NOT common to see gross malnutrition on the streets of our cities but occasional cases are still brought to hospitals and a considerable number of cases develop in our hospitals as a result of other diseases.

Protein nutrition in surgical patients has been the subject of continuous interest in the Department of Surgical Research at the University of Pennsylvania for over 15 years and I shall necessarily present the subject largely in the light of the various problems which my colleagues or I have studied. Many individuals have participated in this program, especially Doctors Ravdin, Riegel, Vars, Koop, Drew and Gimbel.

The work has fallen into several phases. The first phase which I wish to present is an accumulation of data on the protein losses of surgical patients. The next phase was a study of the undesirable effects of hypoproteinemia. The yardstick was the serum protein concentration. Some attempts were made to mitigate these effects by infusions of various types.

Later, an inquiry into the nutritional effects of the stress response was undertaken and nitrogen balance was used as the yardstick. In this way, the nitrogen and caloric requirements of various patients in the postoperative period were studied and the varying degrees of usefulness of a variety of nutritive regimes both enteral and parenteral were compared. An attempt was made to demonstrate objectively an acceleration of convalescence when nitrogen balance was maintained, but the results were not wholly satisfactory. Finally, certain studies were carried out on the utilization of gelatin and of human serum albumin.

[1] May 1, 1951, Cleveland, Ohio.

Examples of losses of protein measured in surgical patients have been assembled in table 1. Table 1 *A* shows losses that have occurred before any operation has been done. Table 1 *B* shows losses estimated during operation and table 1 *C* shows losses sustained in the postoperative period.

It was known from the work of Cuthbertson (1) and the later work of John Eager Howard (2) that after long bone fractures it was difficult, if not impossible, to achieve nitrogen balance by any ordinary increase in the nitrogen intake. Accordingly, a series of 55 surgical patients were studied for five days after operation on various diets, fed either orally or by tube into the stomach or the jejunum, to see if nitrogen balance could be obtained and at about what level. The nitrogen intake ranged from 30 milligrams per kilogram per day to 630 milligrams per kilogram per day. The results showed that only 2 of 37 patients had a positive nitrogen balance at an intake below 300 milligrams per kilogram of body weight per day of nitrogen with a total of 30 calories per kilogram of body weight per day. However, at these levels and above, 11 of 18 patients were in positive nitrogen balance for the first five days. A sufficient number were in positive balance to make us feel fairly well satisfied that the catabolic effect of a soft tissue operation, even as extensive a one as a subtotal gastrectomy, could be overcome at relatively liberal intakes such as 130 grams of protein and 2100 calories per day for a 70 kilogram individual. Such amounts seemed more than enough for most neurosurgical patients in spite of the damage to the flat bones of the skull which they sustained. When the intravenous route of administration was used

the results were somewhat less satisfactory, as will be shown later.

That the extensiveness of the operation has a considerable influence on the catabolic response was to be anticipated and was borne out to some extent in our figures and also in a comparison of these figures with other studies in the literature (table 2), such as that of Werner, Habif, Randall and Lockwood (3), who concluded that the negative nitrogen balance after such procedures as elective cholecystectomy for chronic calculous cholecystitis, and herniorrhapy, could be adequately accounted for on the basis of the diminished nitrogen intake at the time of operation and shortly afterwards.

It was suggested by Drs. Koop and Drew (4) that it might be possible in some groups of patients to give them so much food before operation that no special measures would be necessary to keep them in positive balance after operation and yet to have them remain in positive balance when the entire period was considered. Accordingly, a group of patients was very actively fed for five days before operation, the amounts ranging up to 800 milligrams of nitrogen per kilogram per day with a liberal caloric intake. In order to get this amount of food into the patient, concentrated forms of food nitrogen were usually required, such as amigen powder, which were frequently mixed into an emulsion and pumped through a gastric tube into the stomach. We were interested to learn that patients who appeared to be in normal nutritional condition would apparently retain substantial amounts of nitrogen on such regimes. With one exception, the amount retained for five days before operation always exceeded the amount of the net loss in the five day postoperative period during which no special measures were taken. The results of these preoperative feeding experiments are shown in table 3.

The very basic question of whether maintenance of nitrogen balance improves the convalescence of surgical patients has been one of the hardest to answer. Gross degrees of undernutrition in the protein field with resulting reduction in the concentration of circulating plasma protein have definite unfavorable effects, which have been clearly demonstrated.

However, the question of whether really excellent nutrition is better than just fair nutrition is a harder one to answer. In an OSRD project, under which most of our nitrogen balance studies were carried out, this aspect was investigated by Dr. Isaac Starr and his associates (5). They approached the problem from a number of points of view. For instance, they tested the metabolic exchange of patients during

TABLE 1. NITROGEN LOSSES IN VARIOUS TYPES OF SURGICAL PATIENTS

	N LOSS gm.	PERIOD days	GM. NITROGEN Min.	Av.	Max.
A. Losses Before Operation					
Bleeding peptic ulcer (16)...	90.72	5			
Thermal burn (17)					
Surface oozing.....	7.84	1			
Total N loss.....	30.88	1			
Small bowel obstruction (18).....	11.04	1			
Long bone fractures (1).....	137.00	10			
Long bone fractures (2).....	190.00	variable no. weeks			
B. Losses During Operation[1]					
Radical mastectomy (19).....			15	24 (4)	31.5
Thyroidectomy.....			3	12 (8)	21.0
Abdomino-perineal resection.....			6	12 (12)	21.0
Gastric operations (complicated).....			9	18 (3)	24.0
Operations involving large body surfaces..			13.5	36 (3)	42.0
Pneumonectomy (20)....			21	57 (16)	108.0
First stage thoracoplasty.			12	22.5 (10)	60
C. Losses After Operation					
Herniotomy (21).....	18.0	10			
Subtrochanteric osteotomy (22).....	65.0[2]	14			
Gastric resection (22).....	54.0	5			
Cholecystectomy (21).....	114.0	10			
Gastric resection (21).....	175.0	10			
Acute appendicitis (peritonitis).....	49.0	10			
Repair, perforated peptic ulcer (21).....	136.0	10			
Radical mastectomy (21)....	15.0	10			

[1] Based on 3 gm. N/100 cc. blood.

[2] Average of 3.

standard bed exercises, and concluded that those who were well nourished did, on the average, manifest greater efficiency in performing such work than those who were malnourished. However, this conclusion applied only to the averages and did not hold well for individual cases.

Later, Dr. Starr and his collaborators did a considerable series of ballistocardiographic studies on these patients. They analyzed these records for abnormal complexes and found that the patients that had been maintained in positive balance showed fewer such abnormal complexes than did those who were in negative balance. Statistical analysis of the data obtained showed that it was not quite statistically significant. There were about eight chances out of 100 that the results could have been explained by chance instead of the accepted five or less.

TABLE 2. ESTIMATED NITROGEN REQUIREMENTS FOR EQUILIBRIUM IN EARLY POSTOPERATIVE PERIOD

	N gm/kg/day	CAL. kg/day
Gastrectomy (22)	0.30	30
Craniotomy (23)	0.25	25
Gastrectomy (24)	0.40	16
Gastrectomy (25)	0.39	57[1]
Herniorrhaphy (24)	0.21	not stated
Cholecystectomy (3)[2]	0.20	35

[1] Strongly positive balance.
[2] Calculated from chart on assumption that patient weighed 70 kg.

Good objective evidence indicating that patients kept in positive nitrogen balance after operation are significantly better off than those that are allowed to go into mild negative balance has not been advanced, so far as the author is aware. However, many clinicians have stated unequivocally that they thought that the patients who were well nourished did convalesce better, that they had fewer complications, that they were able to be out of bed earlier, and that they were able to go home from the hospital earlier. Pending more precise demonstration of this result, it is perhaps safe to accept these clinical impressions as a working hypothesis and to feel that it is worthwhile to pursue methods of keeping patients in positive balance through severe illnesses and after operation.

There is, however, another school of thought, expressed particularly by Dr. Francis Moore (6) of the Peter Bent Brigham Hospital, who takes the point of view that because the catabolic response is characteristic of the well nourished individual and not of the malnourished individual it is a teleological mechanism, and that perhaps we should not interfere with it. Unfortunately, even if one accepts the hypothesis that it is a teleological mechanism, it is not evident whether it is to the advantage of the organism to get rid of this labile protein or whether it is to the advantage of the organism to have this labile protein circulating in the body. If the former is the correct explanation, it would seem unwise to attempt to supply protein during the catabolic phase but, if the latter is the correct explanation, it would perhaps be all the more reason to supply protein vigorously so that this catabolic response could be kept up as long as the organism required it, rather than to permit it to go on to exhaustion after a relatively short period. Here again, I believe we have no definitive answer but there are suggestive data in the field of liver injury. Here, Ravdin, Vars and Goldschmidt (7) showed that the degeneration and necrosis in the liver of the rat following an hour's anesthesia with chloroform was decreased if the protein reserves were augmented, and was also decreased if the catabolic response was called out by the subcutaneous injection of an irritating substance such as sodium ricino-

TABLE 3. NITROGEN BALANCE STUDIES IN 11 PATIENTS RECEIVING FORCED FEEDING FOR 5 DAYS BEFORE OPERATION[1]

PATIENT	OPERATION	10 DAYS	FIRST 5 DAYS	SECOND 5 DAYS
		gm.	gm.	gm.
RI	Craniotomy	+92.0	+148.0	−56.0
FE	Craniotomy	+65.4	+134.2	−68.8
SM	Gastric resection	+81.1	+110.1	−29.0
HO	Craniectomy	+39.9	+68.1	−28.2
WA	Rankin resection	−3.1	+57.6	−60.7
DO	Rankin resection	+19.9	+80.9	−61.0
PA	Gastric resection	+68.6	+113.5	−44.9
TH	Craniotomy	+46.0	+71.4	−25.4
WI	Craniotomy	+74.7	+86.9	−12.2
BO	Craniotomy	+113.3	+138.1	−24.8
SC	Craniotomy	+64.8	+98.5	−33.7

[1] After KOOP *et al.* (4).

leate. These experiments would seem to suggest that the availability of the mobilized protein was of advantage in reducing the susceptibility of the liver to injury. Such evidence certainly suggests that, from the standpoint of liver injury at least, it would be worthwhile to maintain the intake of protein at a high level striving for nitrogen balance even during the so-called catabolic phase.

The next phase of the subject which I wish to discuss is the methodology of providing the appropriate protein intake. Where food can be ingested and utilized by the oral route, I think that no one questions that this is preferable. However, patients who have this capacity are not often the ones who develop protein deficits. Instead, the protein deficits are usually seen in patients with gastrointestinal lesions or other patients who find it difficult or impossible to take adequate amounts of nutriment by mouth. As much as we may dislike it, therefore, it is frequently necessary to turn to other routes of administration. In patients in whom anorexia is the only difficulty about using the oral route, it is frequently possible to increase protein intake by improving the palatability of the food by the use of carminatives, by the use of bile salt preparations in patients in whom bile salts are not being supplied to the alimentary tract in normal concentrations, by the use of such vitamins as thiamine where these are not present in sufficient quantity, possibly by the use of small doses of insulin before meals, and finally by tube feeding where this is tolerated. Tube feeding has been made more practical and more comfortable by the use of small plastic tubes to take the place of the 5–6 millimeter rubber tubes used so frequently in the past.

When only the upper alimentary tract such as the esophagus and the stomach is diseased, it is sometimes possible to nourish the patient by jejunal feeding and, in the case of gastrectomy patients, if difficulty with nutrition is anticipated, a two lumen tube may be placed at the time of operation which will permit feeding into the jejunum at the same time as suction is maintained on the stomach to prevent any pressure from being built up behind the anastomosis in this area. While good use of this method has been made in the past, it is seldom necessary in patients who are fairly adequately prepared for gastrectomy. Jejunostomy as a preliminary operation has been suggested by Allen (8) and others and is occasionally carried out. Whether the jejunostomy is done or the oro-jejunal method employed, it must be remembered that the jejunum can tolerate hypertonic solutions very poorly. Frequently, they produce diarrhea with wastage of most of the material administered along with large quantities of fluid and salt. The use of homogenized milk has been advocated for jejunal feedings by Zollinger (9) and his group at Ohio State University, because they have found the smaller fat particles are better tolerated.

The rectal route, while of considerable theoretical interest, has never been of wide practical value. Early in this century, it was used extensively but the preparations administered were probably not absorbed as they consisted largely of various types of milk shakes. With the studies of Abderhalden (10), Folin (11), and others, it was shown that protein foods could be absorbed from the large bowel after they had been hydrolyzed sufficiently and it was further shown that it was not necessary that they be hydrolyzed completely to amino acids. So far, proctoclysis has always been a rather uncertain route of administration even for water and Ebeling (12) showed that it was a very poor route of administration for glucose, only very small amounts being absorbed. All in all, the method has been too uncertain to be of much practical use.

This leaves us with routes such as the intraperitoneal, intramedullary, subcutaneous, and intravenous; in other words, with the parenteral routes. Of these, by far the most useful has been the intravenous route. At present, there are two interrelated restrictions to the successful use of this method. The first is that of venous thrombosis, the second is the fact that we have no reliable fat preparation suitable for intravenous use. While our experience confirms that of Elman (13) that it is sometimes possible for a short period to achieve nitrogen balance without caloric balance, we feel very strongly that caloric balance should be sought and that anything like full use of the proteins given will not be obtained unless the caloric requirement is met. With this in view, if one is to give 30 calories per kilogram to a 70 kilogram individual, it is necessary to provide 525 grams of carbohydrate and protein. Dissolved in 3000 cc. of fluid, this necessitates the use of concentrations of the order of 17 per cent. Concentrations of 15 per cent are the rule in patients in whom an earnest effort is being made to meet caloric and

nitrogen requirements in the early postoperative period. Such concentrated solutions are relatively irritating to the intima of the veins.

In the dog, it has been possible to use highly concentrated solutions for nearly six months at a stretch by advancing a small plastic catheter down the jugular vein until the end of it lies in the superior vena cava where concentrated solutions are discharged into a relatively large volume of blood. While similar methods have been attempted in man, some clot formation about the catheter has at times occurred, and we do not generally feel satisfied to use it, except occasionally in patients who have a very poor prognosis for other reasons. Studies shown in table 4 were carried out in patients who were able to tolerate fairly high concentrations of material by vein for a five-day period. Table 5 gives data obtained with enteral feeding for comparison.

The general conclusions which can be stated from these studies are perhaps the following: *1*) There is a good deal of individual variation in the response of different patients, which of course was expected. *2*) Utilization of protein seems slightly more efficient by the enteral route than by the parenteral route; when the oral or jejunal route was used there seemed to be no advantage to using hydrolysates as opposed to whole protein. *3*) That mixtures of gelatin with the hydrolysate of a complete protein gave slightly better results than when the hydrolysate of a complete protein was used as the sole source was somewhat surprising. This is perhaps explained by results obtained with serum albumin by Dr. Nicholas Gimbel (14). Here better nitrogen balances were obtained when the material was given intravenously than when it was given by mouth. Advantage was taken of the fact that albumin has twice as much sulfur per gram of

TABLE 4. NITROGEN BALANCE STUDIES IN PATIENTS RECEIVING PROTEIN HYDROLYSATES PARENTERALLY[1]

PATIENT	INFUSION	TOTAL N	N	HYDROL. N	CAL.	TOTAL	DAILY
		gm.	*gm/kg/day*	*gm/kg/day*	*kg/day*	*gm.*	*gm.*
TO	Rask AA	60.3	0.26	0.26	34	−11.8	−2.3
KI	Rask AA	86.7	0.28	0.28	39	−21.4	−4.2
MCL	Amigen 5%	90.0	0.30	0.30	19	−27.4	−5.4
DU	Amigen 5%	103.8	0.30	0.30	30	−29.8	−5.9
JA	Casein aminosol 5%	77.9	0.27	0.27	31	−11.9	−2.3
AH	Casein aminosol 5%	97.0	0.30	0.30	36	−1.1	−0.2
GO	Fibrin aminosol 5%	100.5	0.31	0.31	33	−12.7	−2.5
WH	Amigen 5%	64.2	0.20	0.20	31	−0.7	−0.1
HA	Amigen 5%	87.9	0.29	0.29	17	−44.9	−8.9
PO	Casein aminosol	189.0	0.69	0.69	40	+40.4	+8.0
CA	Fibrin aminosol	140.5	0.62	0.62	38	+21.4	+4.2
MCC	Fibrin aminosol	130.5	0.54	0.54	39	+25.4	+5.0

[1] After KOOP *et al.* (26).

TABLE 5. ENTERAL FEEDING AT LEVELS TO COMPARE WITH PARENTERAL FEEDINGS[1]

PATIENT	FEEDING	INTAKE TOTAL N	INTAKE N	CAL.	OUTPUT TOTAL N	BALANCE TOTAL N 5 DAYS
		gm.	*gm/kg/day*	*kg/day*	*gm.*	*gm.*
BE	Amigen	78.7	0.27	26	65.3	+13.4
SC	Gastrostomy	58.5	0.27	32	61.3	−2.8
PO	Gastrostomy	64.5	0.28	31	72.7	−8.2
DO	Amigen—hosp. diet	109.5	0.30	30	106.2	+3.3
BR	Amigen—hosp. diet	88.2	0.30	33	77.1	+11.1
CL	Amigen	75.0	0.30	33	69.6	+5.4
GA	Amigen	100.0	0.30	34	81.4	+18.6
CH	Lactalbumin hydrolysate	70.5	0.30	36	54.1	+16.4
BA	Lactalbumin hydrolysate	98.0	0.30	37	91.1	+6.9
TO	Gastrostomy	73.6	0.34	34	68.8	+4.8
BRU	Gastrostomy	160.0	0.63	35	134.8	+25.2

[1] Ref. 26, table 2.

nitrogen as does casein and most of the other body proteins. Studies of the sulfur excretion and nitrogen excretion, therefore, served as an index of when albumin was being metabolized and when other protein was being metabolized. This indicated that albumin was being stored when given parenterally. Additional data and confirmation of this were obtained by cannulating lymphatics in the extremities of one subject who proved to have three grams per cent protein in his lymph and nearly all of this protein was albumin. Thus, it became evident that albumin was stored in significant amounts, not only in the plasma but also in the interstitial fluid, although no storage of this material was evident in histologic studies of tissues.

Better controlled studies of the metabolism of gelatin administered intravenously in conjunction with a hydrolyzed complete protein were carried out in dogs by Martin Rhode and his associates (15). These indicated that there was perhaps a 27 per cent utilization of the gelatin protein.

At present, a fairly standard mixture for parenteral alimentation would seem to consist of approximately 10 per cent glucose and 5 per cent of an hydrolysate of a complete protein such as casein or fibrin. Three liters of such a mixture provides 1800 calories and 4 liters provides 2400. The volume can be adjusted somewhat to the predicted requirements of the individual. It is generally necessary to change the site of injection at least once in every 24 hours. The spillage in the urine is not a serious factor if the administration is carried on slowly and more or less evenly throughout the 24 hours. With more rapid administration, a larger percentage of the material will be lost. Providing the quantity of salt is held down to the actual requirement of the patient and administration is slow, the probability of pulmonary edema from overloading the circulation is not large, though this may occur in people with unusually poor circulatory apparatus or people with fairly good circulatory apparatus if the rate of infusion becomes too rapid.

At present, I think we can say that parenteral alimentation adequate to meet caloric and nitrogen requirements is possible in perhaps two-thirds or three-quarters of postoperative patients, but that in a smaller group of individuals venous thrombosis occurs so readily that it is impossible to carry out a program of parenteral alimentation in the quantities needed for nitrogen balance. In such individuals, however, the degree of negative balance can often be diminished by giving smaller amounts of the nutrients.

I believe all of this discussion points out the great importance of developing a suitable stable preparation of emulsified fat for intravenous use. The high caloric value of fat, coupled with its high molecular weight and low oncotic pressure make it possible to give a patient a complete caloric ration intravenously with the use of only midly hypertonic fluids. It is to be hoped that further investigation will be carried forward along this line at the same time that nutritional research is going forward in other directions, because it seems likely that this one additional agent, already realized except for stability, would make possible a really adequate means not only of achieving nitrogen equilibrium by the parenteral route, but of achieving a large enough positive balance completely to restore the nutritional deficits of patients with lesions of the gastrointestinal tract before operation.

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